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MASTER THESIS

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Study of treated and untreated oil based drilling waste using a biomarker approach : Gill and liver histopathology in Atlantic salmon (*Salmo salar*).

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ABSTRACT

In order to safely dispose and use treated drill cuttings waste oil based mud, it is important to obtain knowledge about the contamination levels for possible adverse effects in freshwater organisms. Selected biomarkers were studied in Atlantic salmon (*Salmo salar)* exposed to treated and untreated drill cuttings waste (OBMs). The fish were exposed for 14 days in a continuous flow system to nominal concentrations of 0.1 and 1 ppm drill cuttings waste. Sampling was done 3 times during the exposure period but only samples at 14 days of exposure were object of histopathological analysis. Polycyclic aromatic hydrocarbons and heavy metal content in the freshwater were analysed at the end of the exposure period. Histopathological biomarkers of effect were studied then in gills and liver of fish by means of image analysis. Results showed that gills and liver were severely damaged with the high untreated group (1 ppm) to a lower extent with the high treated group and no considerable effects with the control. Dunnett's test was performed (only for gills data) to test and highlight the significant difference between exposed groups in comparison to the control. The affected gills were mostly damaged by aneuryisms, epithelial lifting and necrosis probably due to constituents of the mineral oils or heavy metals. The lack of proofs of the liver data made statistical analysis impossible for the liver histopathology. Particularly noteworthy is the sensitive response of this high order biomarker of effect compared to those at lower organisation level in this study, and compared to similar responses in another study where salmon exposed to crude oil.

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LISTE OF ABBREVIATIONS

WBFs : Water based fluids

OBFs : Oil based fluids

SBFs : Synthetic based fluids

TCC : Thermomechanical Cuttings Cleaner

PAHs : Polycyclic aromatic hydrocarbons

EPA : Environmental Protection Agency

TPH : Total Polycyclic aromatic Hydrocarbon

PCBs : Polychlorinated Biphenyls

PBBs : Polybrominated Biphenyls

FCA : Foci of cellular alteration

NP : Nuclear pleomorphism

MH : Megalocytic heptosis

OSPAR : Oslo-Paris convention

IRIS : International Research Institute of Stavanger

SOP : Standard Operating Procedure

FFW : Filtered fresh water

TDC : Treated drill cuttings

UDC : Untreated drill cuttings

LSI : Liver somatic index

CF : Condition factor

EH : Epithelial hyperplasia

LF : Lamellar fusion

BC : Blood congestion

EMS : Excess mucus secretion

PEC : Proliferation of epithelial cells

An : Aneuryisms

EL : Epithelial lifting

LC : Lamellar clubbing

Nec : Necrosis

GST : Glutathione S-transferase

EROD : Ethoxyresorufin O-deethylase

LMS : Lysosome membrane stability

1. INTRODUCTION

Petroleum drilling is the primordial step in the success of oil field exploration. Drilling fluids are commonly used and represent one fifth between 15% and 18% of the total cost of well petroleum drilling. They must generally comply with three important requirements: i) easy to use, ii) not too expensive and iii) environmentally friendly (Khodja et al., 2010). Drilling fluids are used in large quantities to optimise on- and off-shore drilling operations (Neff, 2005).

Oil well drilling fluids are used during operations. A rotating drill bit that is attached down of a drill pipe breaks off small pieces of rock called drill cuttings. These cuttings go up along the drill string as the drilling fluids are pumped down. The fluids itself have a property that cool the drill bit and maintain pressure control of the well as it is being drilled (Melton et al., 2004).

The composition of drilling fluids is based on a mixture of clays and additives in a base fluid. There are three generic types of base fluids such as water based fluids (WBFs), oil based fluids (OBFs) and synthetic based fluids (SBFs) (Sadiq et al., 2003).

Among these fluids, WBFs are the most commonly used, relatively economical and easy to dispose of because they are biodegradable and considered as very low toxicity so their disposal on- and off-shore is never shown any harmful effect (Soegianto et al., 2008). Discharges of contaminated drill cuttings (mainly OBFs) have in the past caused considerable change of the benthos adjacent to many oil and gas platforms in the North Sea. In strongly affected areas, the fauna is of low diversity and dominated by opportunistic species (Schaanning et al., 2008).

Due to the environmental issues caused by these contaminants that come along with the drill cuttings, a so-called Thermomechanical Cuttings Cleaner (TCC) method has been developed to treat the oil based drill cuttings before any uses or disposal. This method has a purpose of getting rid of the oil that is adsorbed on cuttings. It aims to reduce as low as possible the toxicity of the hydrophobic compounds or even transforms the drilling wastes into material inert for the environment. Mainly, the 16 polycyclic aromatic hydrocarbons (PAHs) recognised by EPA as potentially carcinogenic compounds are the unwanted chemical and represent a target for decontamination of drill cuttings.

Although the cuttings are cleaned, a question is still on-going like “how clean is clean enough?” In this context, this thesis focuses on the evaluation of toxicity of treated and untreated drill cuttings using a test organism Atlantic salmon (*Salmon salar)*.

This thesis studied histological changes in Atlantic salmon tissues when exposed to drill cuttings within 2 weeks period.

1.1. Drill cuttings related oil based mud

➲ Description

According to Neff et al (1987) drill cuttings are particles of crushed rock produced by the grinding action of the drill bit as it penetrates into the earth. Drill cuttings range in size from clay-sized particles (~ 2 μm) to coarse gravel (> 30 mm) and have an angular configuration (Neff, 2005). The drill cuttings themselves are safe so they do not present any harmful effect to the environment but the adherence of toxic pollutants that are present in the drill fluids (also known as mud) make them relevant for environmental studies.

➲ Composition

Drill cuttings contain, in addition to formation solids, small amounts of liquid and solid drilling mud components. The amounts of drilling fluid solids that remain attached to cuttingsvary, depending on the grain size of the crushed rock from the strata being drilled (Neff, 2005).

In this study, the drill cuttings related OBMs were analysed prior to the main experiment. This includes treated and untreated drill cuttings provided by Halliburton, a service company. These drill cuttings were analysed prior to the exposure to provide data about its pollutant contains such as TPH (Total Polycyclic aromatic Hydrocarbon), PAH and metals (appendix 1). The chemical composition of drill cuttings reflects the geochemistry of the formation being drilled and the amount of drilling mud ingredients adhering to the cuttings at the time of disposal (Neff, 2005). Several types of metals were reported either in drilling mud and cuttings such as cadmium, chromium, copper, mercury, nickel, lead, and zinc. The amount of these metals tends to be not the same from one place to another.

1.2. Drill cuttings disposal options

The amount of drill cuttings that is being produced depends on the type of the base fluid. For example, the uses of WBMs generate between 7000 and 13000 barrels (bbl) of waste per well. Depending on the depth and diameter of the well, about 1400 - 2800 bbl of that amount are drill cuttings (Soegianto et al., 2008). As opposed to that, OBMs generated more than 13000bbl which made them more efficient just because of their good properties to (i) stabilise the well-bore, (ii) give a better lubricity between the drill string and the borehole, (iii) to have a high temperature stability, (iv) to prevent hydrate formation and, (v) to provide

a high viscosity (Melton et al., 2004). As part of the drilling process, offshore drilling wastes are brought to land where they are treated and processed for mud recovery by leaving the drill cuttings free of mud. Three main solids waste disposal options can be done according to the oil and gas company choice with regards to cost-benefit. It includes offshore discharge, offshore re-injection and onshore disposal (safe storage, potential use).

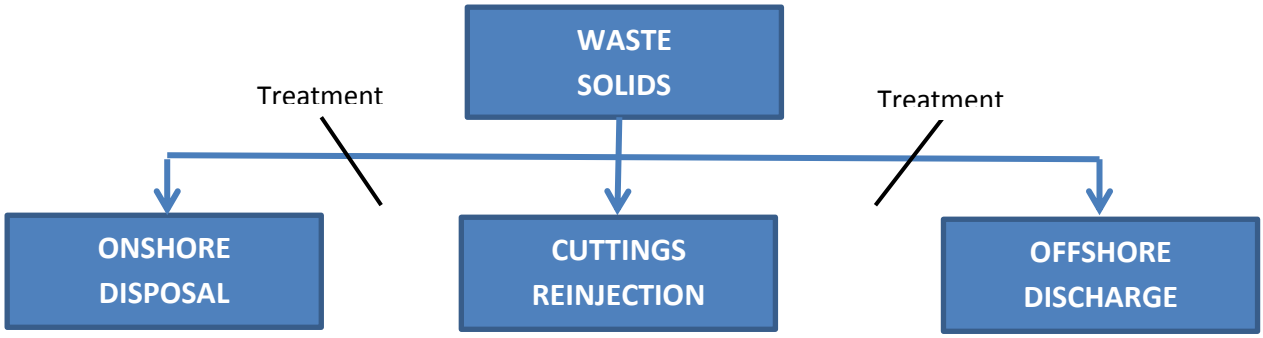


Figure 1: Flow chart showing all the possible options for drilling waste management.

1.3. Environmental issues related to drill cuttings disposal

This present study was done based on the Poland project untitled "*Conception of reuse of the waste from onshore and offshore drilling in the aspect of environmental protection*", that is in cooperation with the University of Stavanger. The objective of the Poland project is to set methods and processes allowing for the transformation of drilling waste into inert materials and also the development of comprehensive method of onshore and offshore drilling wastes management. Hence wastes generated in course of the onshore and offshore drillings are frequently contaminated with toxic substances, particularly of the type of petroleum derivatives, heavy metals, radioactive compounds, reductive processing of organic compounds product and the environmental ecotoxicology study of the treated drilling waste is relevant.

Operators are interested in bringing drill cuttings to land-fields for treatments and disposal. As it is treated and disposed, it might have a use in several purposes such as agriculture additive, construction or dumped into the ground. The cleanness of the drill cuttings depends on the efficiency of the method that is used. Although they are treated, they still contain a low amount of toxic compounds which if not well-controlled can contaminate the surrounding environment. Rain and water runoff are the most common environmental parameters that lead to spreading of ground water contaminants. As water goes through the soil layers along with the drill cuttings it changes the soil structure by translocation of materials. Chemical pollutants that adhere to the cuttings can be moved and deposited from

one layer to another by the natural occurring processes called “eluviation and illuviation” (Holden, 2005). Those contaminants may go through the soil layer and be added to the ground water where they may further be washed out into rivers, lakes and the sea. Metals will partly be dissolved into the ground water and form solutes while the hydrophobic compounds will largely remain adsorbed to particles. In the course of such spreading processes many fresh water organisms may be exposed to these contaminants in their habitats. This could be by exposure of fish directly, or it could be potentially taken up via the food chain. The Atlantic salmon was chosen as target species for the study to evaluate the possible toxic effect of the drilling wastes.

1.4. Atlantic salmon (*Salmo salar*)

➲ Description and classification

The Atlantic salmon (Salmo salar) is a member of one of the most primitive superorders of the Teleosts or bony fishes, namely the Protacanthopterygii, which includes the Salmonoids and a few genera of deep-sea fish (Mills, 1991). It belongs to Salmonidae family (Klemetsen et al., 2003). Salmonidae includes the Atlantic and Pacific salmon, the trout and the charr, classified as the Salmoninae that comprises about 30 species of fish in seven genera according to Behnke (1991) and Nelson (1994) of which Salmo, Salvelinus along with Oncorhyncus are the best studied. They represent a subject of interest to study the effects of geographic or physiologic isolation (Evermann, 1925).

➲ Distribution

The life cycle, migration and distribution of Salmo salar are very intricate. Geographically, they are found in river systems on both sides of the Atlantic and migrate over most of the northern part of the ocean during anadromy (Hansen & Quinn, 1998). In the age of parr where they have a fresh water life cycle, they are very common throughout the entire distributional range in North America (Power 1958; MacCrimmon & Gots 1979). In Europe, they are normally associated with larger lakes (MacCrimmon & Gots 1979; Berg 1985; Kazakov 1992), although several resident riverine populations exist (Berg & Gausen 1988).

➲ Morphology and anatomy

There are a number of anatomical features which help in the identification of the various salmon species. Those used by taxonomists include scale and fin ray counts and the number and shape of the gill rakers on the first arch (Mills, 1991).

Table 1. General distinguishing features of salmon modified from Mills (1991): "Distinguishing features of species of the genus *Salmo*".

Atlantic Salmon (*Salmo salar*)	
Upper jawbone	Extends to the level of the rear of the eye
Scale count between base of adipose fin and lateral line	10 – 13
Number of dorsal fin rays	10 - 12
Number of anal fin rays	8 – 11
Number of gill rakers on first arch	15 – 20 (slender)
Other distinguishing features	Caudal peduncle narrow; caudal fin shallowly forked

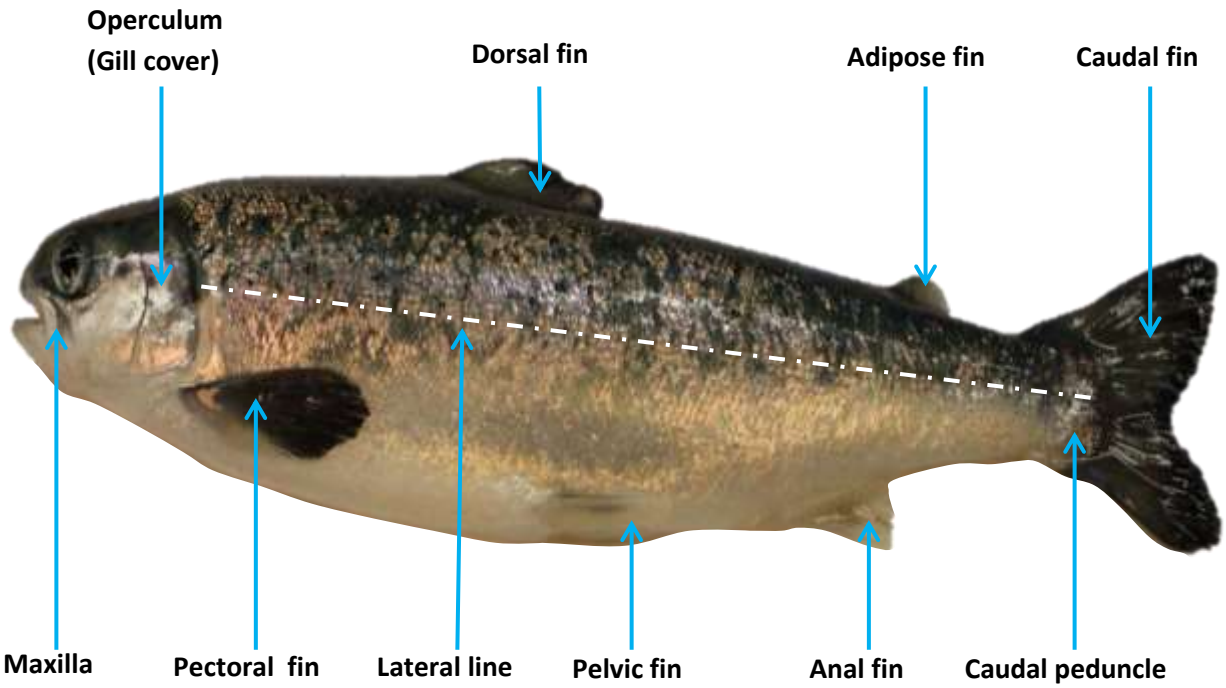


Figure 2: Main morphological features of Atlantic salmon (*Salmo salar*)

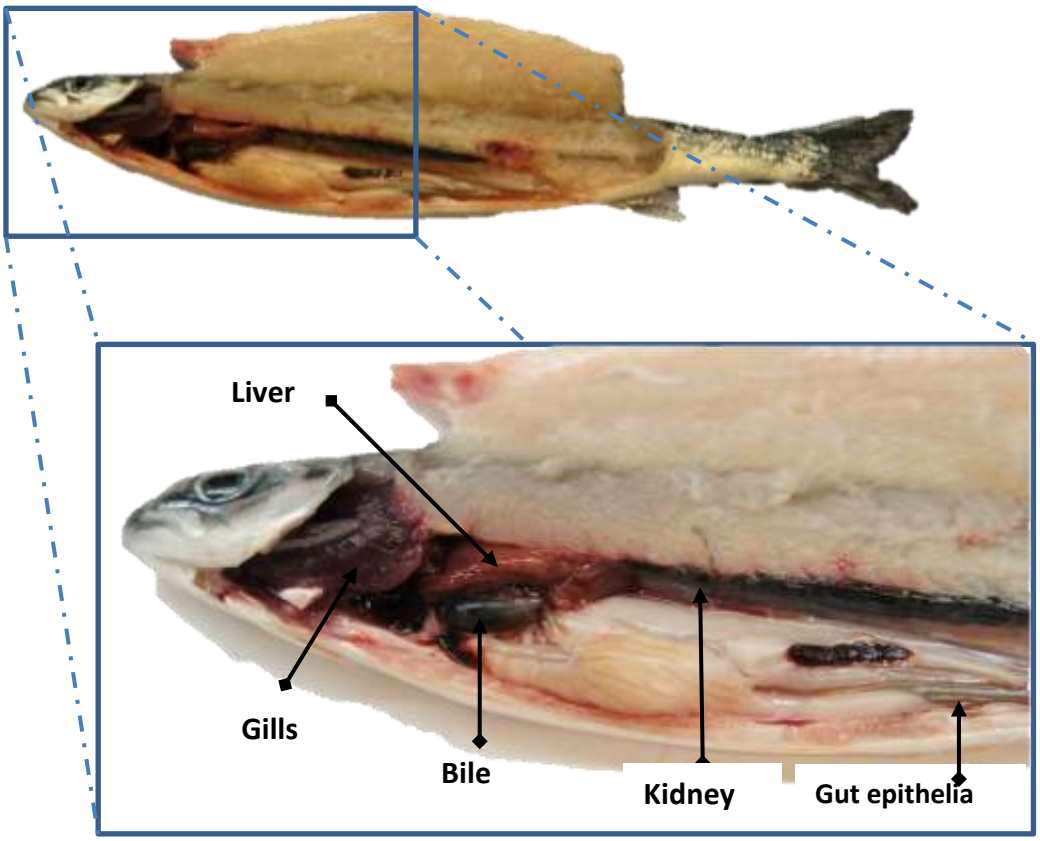


Figure 3: Internal anatomy of a typical individual of Atlantic salmon from our study.

➲ Atlantic salmon life cycle

The life cycle of Atlantic salmon is split in two different parts: they first spend their lives in fresh water where at a certain age (after age of parr), they undergo physiological changes known as smoltification. Besides, the adults as four year old will return to the river where they were hatched to spawn. Unlike Pacific salmon, Atlantic salmon may repeat the spawning migration.

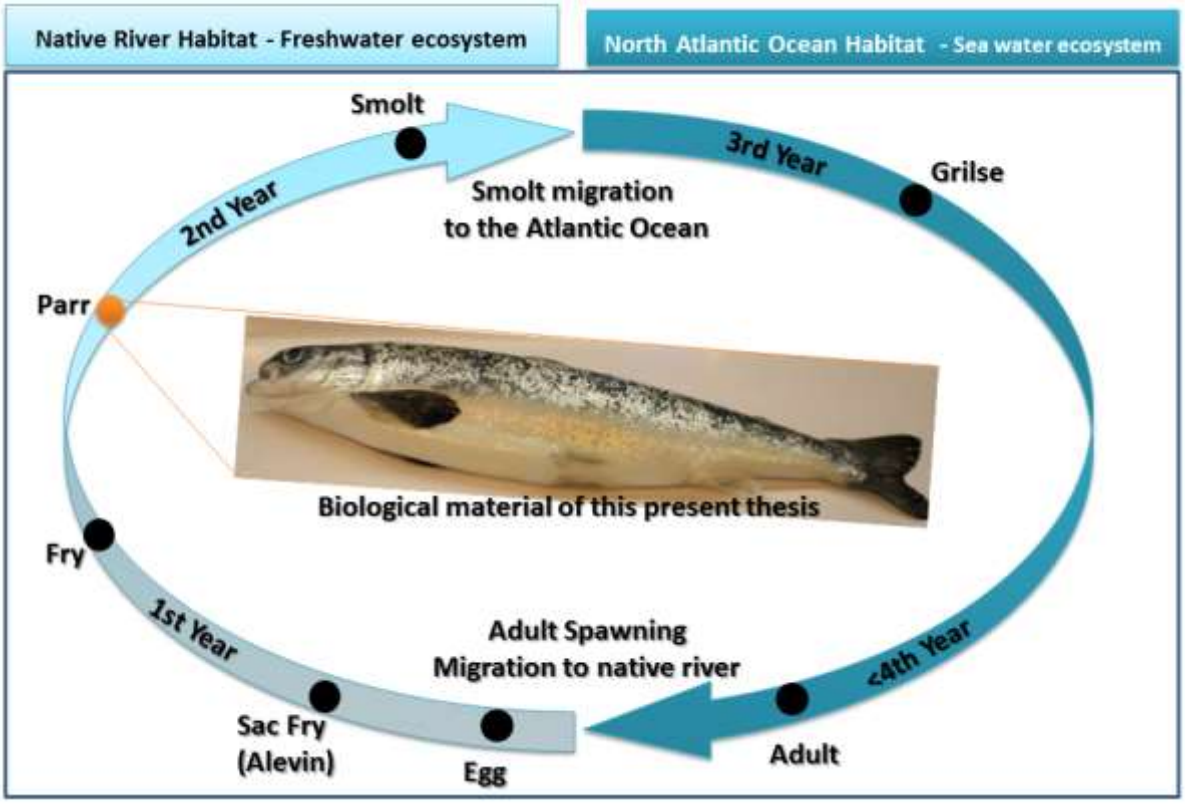


Figure 4: Atlantic Salmon life cycle

As shown with a single red dot in the figure 4, parr is the stage between fry and smolt where they do not have yet a defined gender. One particularity that defines a parr is the vertical markings called “parr marks”. They spend their life in freshwater and migrate to the sea water after age of smolt. They remain in the river for 2 to 6 years depending on water temperatures and food availability. Parr was used as a biological material of this study to assess the effect of the drill cuttings on fish component of the freshwater ecosystem. It is chosen a fresh water stage of the salmon to represent fresh water organisms, but it is considered in the project that it could also have relevance to effects in marine fish. The present results are compared to a dispersed oil exposure of salt water adapted Atlantic salmon.

1.5. Biomarkers

When contaminants are released to the environment, living organisms are affected by the stressors interfering with and possibly disturbing their bioenergetics balance (Adams et al., 1993). Stressors tend initially to affect the organism at low levels of organisation such as molecules and enzymes (Sherry, 2003). Environmental monitoring is commonly applied to evaluate the uptake of xenobiotics and its potential impact on living organisms (Livingstone, 1993).

The term biomarker has been defined by various authors. According to Walker et al (2012), a biomarker is defined as any biological response to an environmental chemical at the individual level or below demonstrating a departure from the normal status. Thus biochemical, physiological, histological, morphological, and behavioural measurements are considered biomarkers. The National Academy of Sciences in the United States defined biomarkers as "a xenobiotically induced variation in cellular or biochemical components or processes, structures, or functions that is measurable in a biological system or sample (National Research Council, 1987; Connell et al., 2009)". It can be seen also as "any biological measurable response from an organism, induced by the exposure to a xenobiotic or complex mixture of them" (Sedeño-Díaz and López-López, 2012).

➲ Seeing those definitions, a biomarker can be summarized simply as "***the biological responses to a contaminants exposure***".

According to NRC (1987), WHO (1993) in concordance with Sedeño-Diaz and López-López (2012) and Van der Oost et al (2003), biomarkers can be subdivided in three classes:

- Biomarkers of **exposure**: covering the detection and measurement of an exogenous substance or its metabolite or the product of an interaction between a xenobiotic agent and some target molecule or cell that is measured in a compartment within an organism;

- Biomarkers of **effect**: including measurable biochemical, physiological or other alterations within tissues or body fluids of an organism that can be recognized as associated with an established or possible health impairment or disease;

- Biomarkers of **susceptibility**, which serve as indicators of a particular sensitivity of individuals to respond to the challenge of exposure to the effect of a xenobiotic or to the effects of a group of such compounds, in this case, individual changes included genetic factors and changes in receptors which alter the susceptibility of an organism to that exposure. However, other authors such as Walker et al (2012) stated that a number of classifications of biomarkers have been proposed but the most widely used is division into biomarkers of exposure and biomarkers of effect.

1.6. Biomarker at tissue level

Biomarker can be measured at different level of biological organisation which goes from the molecular to whole organism level. Each of them shows a specific response when exposed to contaminants. In this study, biomarkers have been studied at the tissue level (figure 5) of *Salmo salar*. These biomarkers are attributed to gills and liver and known as histopathological biomarkers. Histopathological biomarkers are valuable as indicators of the general health of fish and can be used to reflect the effects of exposure to a variety of anthropogenic pollutants (Hinton et al., 1992). When a high concentration of chemical pollutants is released in the environment, acute changes can be seen, while for chronic duration information about sublethal aspects of change is required.

One case study listed by Van der Oost et al (2003) in reference to Ortiz-Ordoñez et al (2011) revealed that *Goodea atripinnis* a gadoid fish from Central Mexico showed severe histological damages in gills and liver after a chronic exposure to an herbicide with glyphosate. As result, a lamellar hypertrophy and leukocyte infiltration in gills, and hepatocytes with vacuolization in the cytoplasm and piknotic nuclei in liver were found after 75 days of exposure to pesticide. The same author stated that this pesticide might impair normal organ functioning that could lead to health damage in fish because of the important physiological roles of these organs.

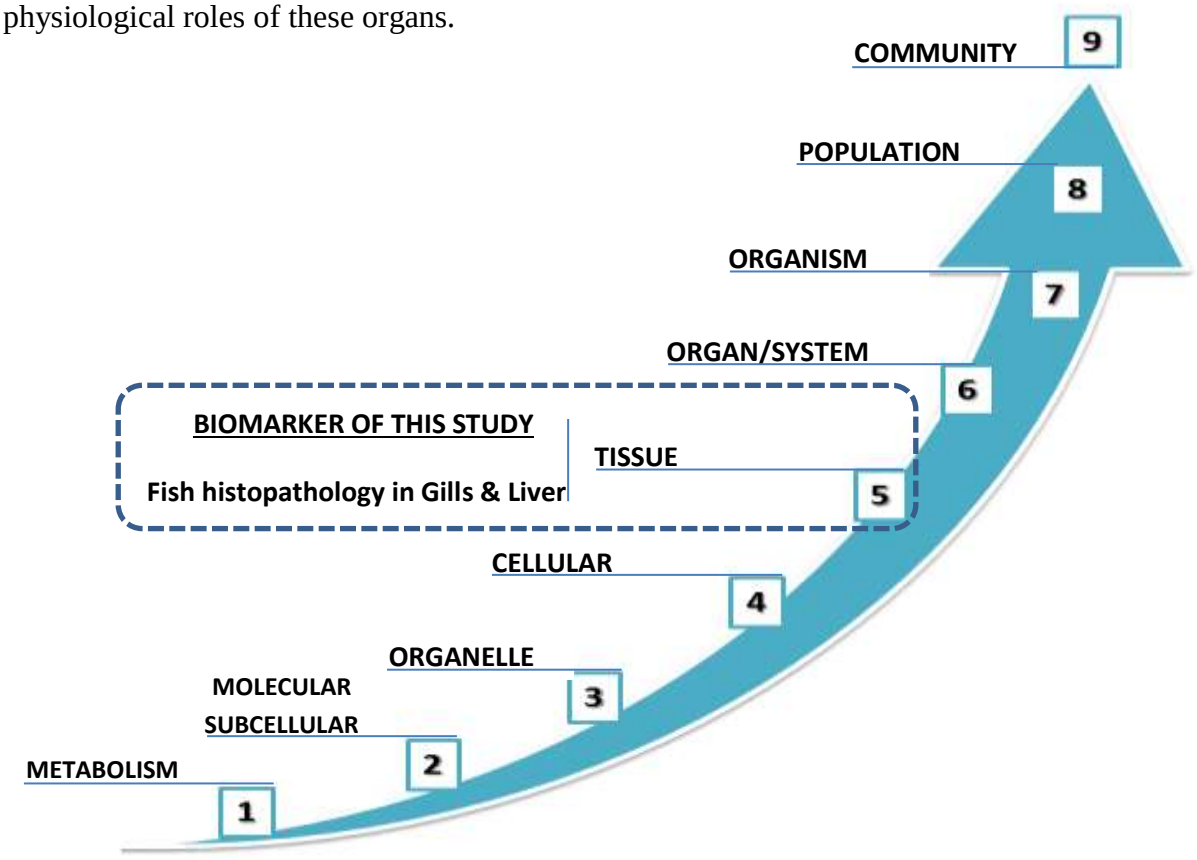


Figure 5: Chart showing the level of biomarkers in relation to biological levels of organization.

Different case studies related to fish histopathology concluded that on a worldwide scale, the most convincing examples of a causal relationship between fish disease and pollution was provided by intensive and detailed studies carried out in North America, particularly on liver pathology (Hinton et al, 1992; Hinton 1994; Vethaak and ap Rheinallt, 1992). Abnormalities such as evolution of cells neoplastic and cancerous diseases in aquatic organisms within their relationship to environmental pollution have been as well critically reviewed by Mix (1986). A certain number of studies tried to link cells abnormalities (example: neoplasia) to the environmental pollution, only in few cases the data supports the pollution and neoplasia relationship (Brown et al., 1977; Kimura et al., 1984; Malins et al., 1985; Myers et al., 1994; Van der Oost et al, 2003).

Methods using quantitative and semi quantitative electron microscopy has been introduced and described by Triebskorn et al. (1997), Schramm et al. (1998) to study liver ultrastructure. Rubberlip surfperch and rainbow surfperch were used and have been exposed to a natural petroleum seep where they revealed a specific sensitivity for histopathology lesions (Spies et al., 1996).

➲ Histopathology of gill

Gill is an important organ for fish since it is multifunctional organ responsible for respiration, osmoregulation, acid-base balance and nitrogeneous waste excretion. This organ is sensitive to chemicals in water, since gill filaments and lamellae provide a very large surface area for direct and continuous contact with contaminants in water.

Mallatt (1985) and Wood (2001) gave comprehensive information on structural changes in fish gills in response to toxicants exposure. Gill alterations are, in general, responsive to contaminant exposure but they are non-specific. The table 2 shows different types of gill alterations in response to contaminants such as organochlorines, petroleum compounds, organophosphates, carbamates, herbicides and heavy metals (Hemalatha and Banerjee, 1997; Global Tox, 1997).

Table 2. Summary of gill histopathology caused by environmental contaminants.

Caused by heavy metals (lead, nickel) and insecticides (PCBs, PBBs).	CAUSED BY CRUDE OIL TOXICITY (Prasad, 1988; Khan, 1995).
Epithelial Hyperplasia with lamellar fusion	Edama formation
Epithelial Hypertrophy	Mucous cell hyperplasia
Telangiectasia	-
Edema with epithelial lifting	-
Epithelial desquamation	-

➲ Histopathology of liver

Teleost liver is the primary organ for biotransformation of organic xenobiotics, and probably also for the excretion of harmful trace metals, food digestion and storage, and metabolism of sex hormones (Health, 1995; Hilton et al., 2001). This organ is suitable for studying environmental contaminant effects since it is very sensitive. Contaminants tend also to accumulate in the liver which makes this organ more exposed to a much higher levels than in other organs (Health, 1995).

A laboratory experiment showed that certain pollutants such as PAHs, aromatic amines, nitroso-compounds are hepato-carcinogens in fish (Moore and Myers, 1994). This liver alteration is a useful indication of chronic toxicity in fish (Bailey et al., 1996; Vandenberghe, 1996). Other alterations indicate the early pathological stages in formation of liver neoplasms including foci of cellular alteration (FCA), hepatocellular nuclear pleomorphism (NP) and megalocytic heptosis (MH) (Simpson and Hutchinson, 1992). The Working Group of Biological Effects of Contaminants (WGBEC) of the International Council for Explanation of the Sea (ICES) proposes that liver diseases are classified as Category II by OSPAR (WGBEC, 2002), and stated that although criteria for which quality assurance procedures are not yet in place they may in spite of that be used for monitoring.

2. MATERIALS AND METHODS

As introduced earlier, Atlantic salmon (*Salmo salar)* was selected as target species. Fishes at the stage of Parr having an average weight (102 ± 17 g) and size (22 ± 1.3 cm) were selected for this study. About 300 fish were purchased from a fish farm in Dirdal on 17th of February 2014 and brought to IRIS-Biomiljø laboratory facility where they were distributed in a five 600 L tanks. Test animals were categorized into treated and control groups (60 animals in each group).

2.1. Acclimation period and daily care

Prior to the exposure, fish were kept in the tank for acclimation (14 days). A filtered fresh water via a carbon filter (Aqua Medic Aktivt kull 4 mm 5 Liter) was flown to the tank with an average flow rate of 4 L/min. Fresh water was supplied using a continuous flow system.

Some parameters were measured and registered on a daily base. These include:

- Oxygen (mg/L) and temperature (^{o}C) using a multi-parameter.
- Water flow (L/min).

In addition to that, feeding and cleaning are also part of the daily care. Commercial fish food was supplied to the fishes during whole acclimation period.

2.2. Exposure set up

This experiment was handled under the SOP procedure untitled "planning experimental activities in the environmental (Biomiljø) pilot hall" at IRIS. The exposure was conducted from 03rd to 17th of March (two weeks). After the exposure finished, one week of recovery time was given to the fish (17th to 24th of March).

A control group was kept without any treatment while the test groups were exposed to untreated and treated drill cuttings. All fish were kept in dechlorinated fresh water. The exposure concentration that was used to make up the exposure solution was prepared according to the following paragraph:

➲ Exposure concentrations:

Two head tanks of 15L (figure 6) were filled with 12L of active coal filtered fresh water (FFW) that is mixed with a defined amount of drill cuttings.

1. Treated drill cuttings (TDC) preparation:

156g of TDC were measured and mixed with 5L of FFW. Altogether, they were added to the header tank where afterwards, 7L of additional FFW was poured to make up the solution onto 12L.

2. Untreated drill cuttings (UDC) preparation:

200g of UDC were measured and mixed little by little with 5L of FFW. The UDC were very difficult to dissolve so they had to be slightly heated and manually agitated using a long spoon acting as a propeller. Once it was well dissolved, the same procedure as used for TDC was applied.

Neoprene tubes were used to link the header tanks with the exposure tanks via two different kinds of pumps. One pump was set at 315 rpm (pump-watson marlow 520s) and used to provide a high concentration of 6.2 mL/min for both treated/untreated drill cuttings and the second (pump- watson marlow 505u) was operated at 62 rpm to produce a low concentration of 2.1mL/min of treated and untreated drill cuttings.

TDC and UDC were continuously mixed by a means of a impeller (heigar EUROSTAR ika Labortechnik) to avoid the settling down of the mud and to keep the solution always in suspension. They were placed upstream of the exposure tanks allowing the gravity to work as a driving force.

Technically, the neoprene tube was attached above the water supply tube in a way that the drilling mud droplets fell down and spread out all over the tank by the FFW jet.

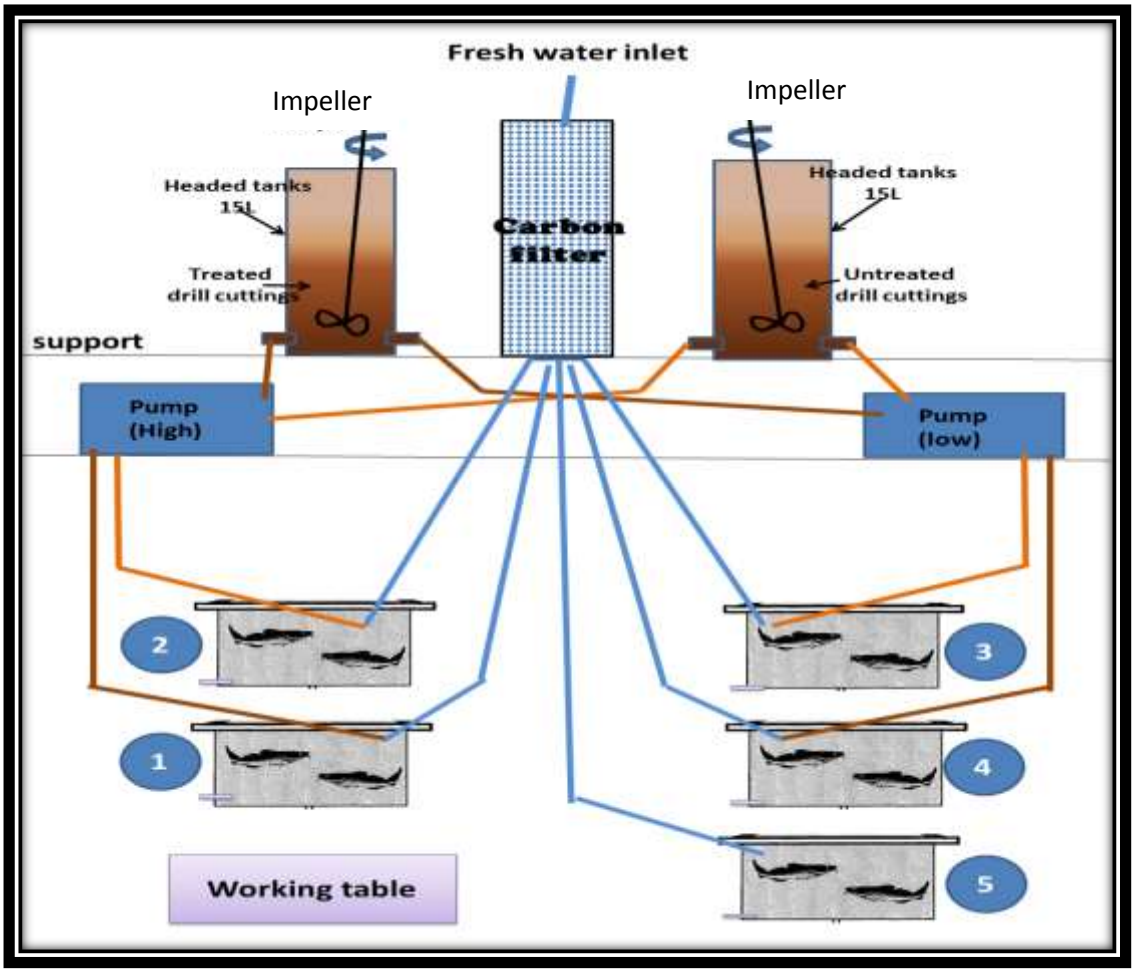


Figure 6: Fish exposure set up

Table 3. Description of the chemical concentration gradient contained in each tank

Tank number	Concentration (part per million – ppm)	Description	Type of added mud
1	1	High	Treated
2	1	High	Untreated
3	0.1	Low	Untreated
4	0.1	Low	Treated
5 (control)	-	-	-

During the exposure period, the fish were not fed. Daily care consisted in:

(i) Measuring regularly the oxygen and temperature.

(ii) Measuring the water flow but just every other day.

(iii) Checking regularly the neoprene tube in case of clogging and/or rupture.

2.3. Sampling

Figure 7 shows details of the sampling including dates, number of sampling and days of sampling from the starting point at time zero (T_0).

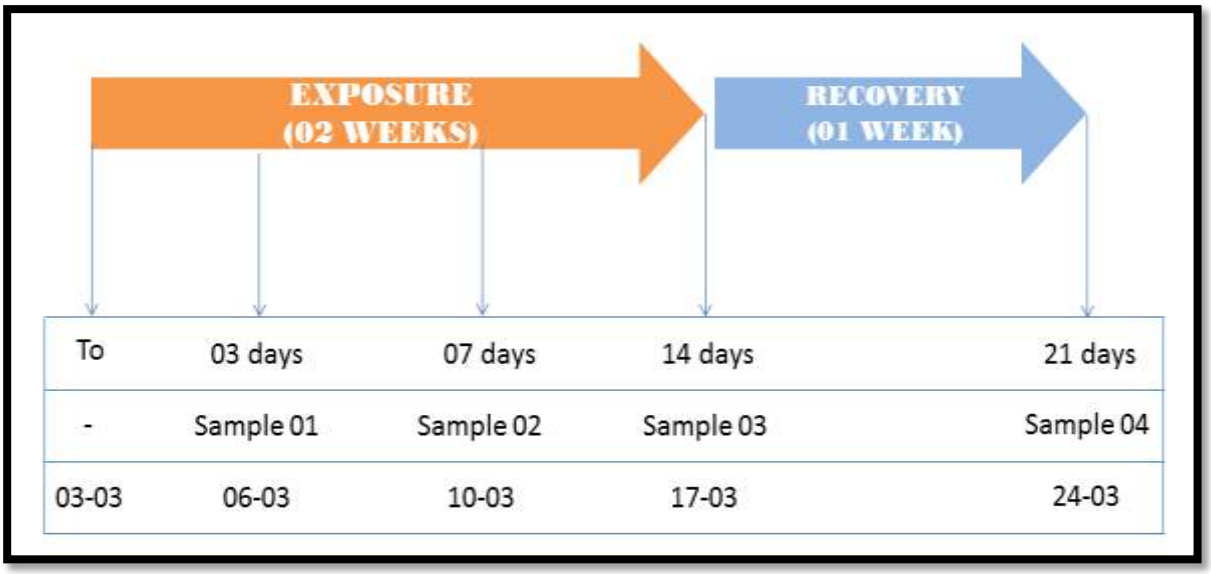


Figure 7: sampling details

On each sampling, 50 individuals were taken from the tanks and sacrificed. Ten fish from each tank were collected by a hove where they are put into a bucket containing 10L of fresh water mixed with anesthetic (50mg/10L of an aquacalm metomidate hydrochloride). By the action of the anaesthetic, fish were immobilized within 3 mins. The table given below summarizes the dose-response of that anaesthetic used for salmonids and other fish (Malmstrøm et al., 1993).

Table 4: Doses for etomidate and metomidate for salmonids, cod and flatfish (Malmstrøm et al, 1993).

Effect	Dose	Induction Time	Max. Exposure Time	Recovery Time
Sedation	3 – 5 mg/L	~ 10 min	Hours	Depends on
Immobilization	5 – 10 mg/L	~ 03 min	Unknown	Exposure time

Prior to the dissection, fish were weighed and length was measured. Afterward, a hammer was used to scarify the fish. Thereafter, they were dissected where the liver is taken out and weighted. Liver was dissected into three pieces for further analysis. Gill samples were also collected and both liver and gill were stored in formalin. After the period of recovery fishes were removed and control as well as treated groups (left over) were killed and disposed in secure garbage.

2.4. Sample preparation for histopathology analysis

The samples were prepared using the following Standard Operating Procedures (SOP) for IRIS Biomiljø laboratory. In order to prevent the appearance of post mortem artefacts, specimens were handled with extreme care. Sampled fish were dissected to take out organs. Analysed tissues (liver and gills) were put in pre-labelled histocassette and placed into histological fixative known as formalin (Baker's calcium-solution: 4% formaldehyde, 1% CaCl2, 2.5% NaCl) for wax sections and stored at 4 ^{0}C until embedding. Chemicals were handled very safely with accordance to the SOP – Safe handling of chemicals in the laboratory.

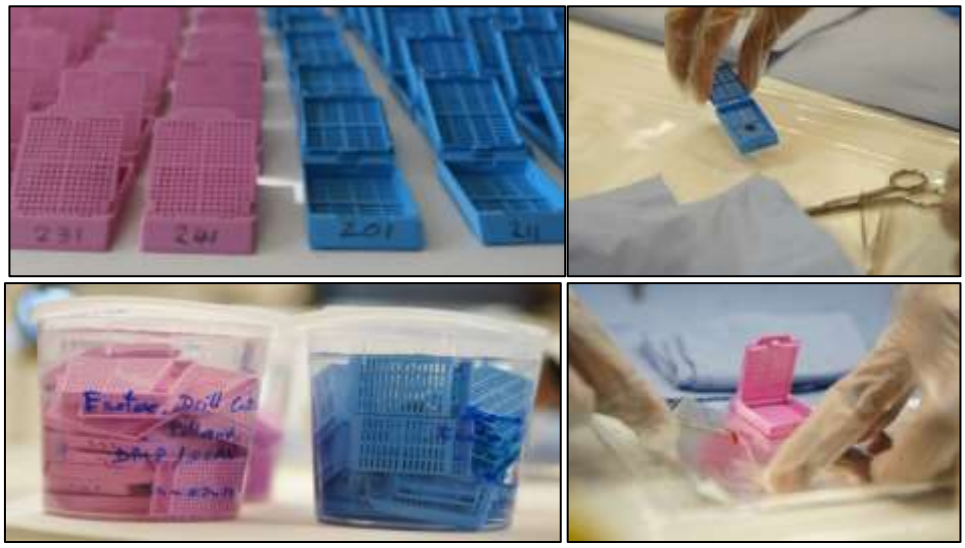


Figure 8: Pre-labelled histocassette, gill and liver sample, histological fixative.

Prior to the embedding, samples (14 days) were distributed into five replicates. One replicate consisted of seven random out of ten samples of fish gills and liver from each group (control and treated).

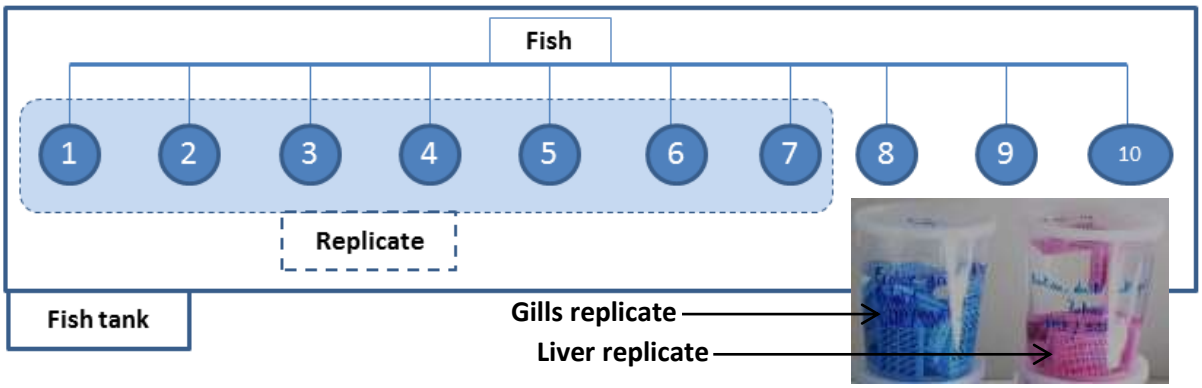


Figure 9: Schematic drawing and photo of a replicate per fish tank.

The embedding was performed at the University Hospital of Stavanger. Tissue samples were dehydrated in alcohols and cleared in xylene ($C_6H_4(CH_3)_2$) and embedded in paraffin. Histological sections (3µm thick) were cut using a microtome HM 355s (Microtom, Bergman), mounted on slides, air dried at 37 ^{0}C for 24 hours and stained with haematoxylin and eosin (appendix 2-staining procedure).

The tissues were examined for health parameters related to histopathological conditions, inflammatory and non-specific pathologies and those associated with pathogen and parasites infections. All micrographs were captured using an Olympus DP72 digital camera mounted on an Olympus BX61 light microscope. All slides were analysed using a histological pictures atlas (appendix 3). Detected histopathological liver lesions were assigned to one of the following groups: steatosis; circulatory disturbance; inflammatory changes; melanomacrophage aggregates; parasites and other pathological changes, according to developed and adopted scoring system while gill alterations were attributed to epithelial lifting, aneuryisms, lamellar clubbing, lamellar fusion, hypertrophy, blood congestion, necrosis, epithelial hyperplasia, excess mucus secretion and proliferation of epithelial cells.

Vacuolation condition, macrovesicular and microvesicular steatosis were distinguished based on the size and the pattern of vacuoles present. Circulatory disturbances included various changes in normal structure of blood vessels (congestion, dilatation, peliosis). Non-specific lesions were presented as: inflammatory changes (lymphocyte infiltration and granulomatosis); melano-macrophage aggregates, parasites, other pathological changes (degenerative – necrosis, proliferative-fibrosis, cirrhotic changes).

According to the affected area or prevalence of each disorder within a specimen, all of the parameters were scaled using an established scoring system (tables 5a and 5b).

Table 5a. Categories for the histological liver lesions and scoring system used for their quantification.

Steatosis (normal cyclical, non-pathological status of the liver)	0 – 3	0-absent 1-area affected 2-some areas affected 3-distributed through the whole sampled tissue
Circulatory disturbances	0 – 2	0-absent 1-sporadic/small area affected 2-some areas affected
Other pathological changes	0 – 2	0-absent 1-sporadic 2-multiple/widespread
Inflammatory changes	0 – 2	0-absent 1-sporadic 2-multiple/widespread
Melano-macrophage aggregates	0 – 3	0-absent 1-area affected (1-2 cases) 2-some areas affected/more than 2 in a sample 3-distributed through the whole sampled tissue
Parasites	0 – 1	0-absent 1-area affected

Table 5b. Categories for the histological gill lesions and scoring system used for their quantification supported by colour difference.

Gill alterations	Score	Meaning
Aneuryisms (An) Epithelial lifting (EL) Epithelial hyperplasia (EH) Lamellar fusion (LH) Lamellar clubbing (LC) Blood congestion (BG) Excess mucus secretion (EMS) Necrosis (Nec) Proliferation of epithelial cells (Pec)	**0 – 4**	**0 : normal** **1: mild** **2: mild to moderate** **3: moderate** **4: severe**

2.5. Support parameters

➲ Liver Somatic Index (LSI)

Liver somatic index was calculated according to the following formula (Sadekarpawar and Parikh, 2013):

$$\textbf{LSI = (Liver weight (g) / Fish weight (g)) × 100}$$

➲ Condition Factor (CF)

Condition Factor (CF) for fish has been proposed by Bagenal and Tesch (1978) and it is based upon the ration between body weight and length. It is stated that this factor may be affected if the availability of food is limited or if the food consumption of the fish is impaired due to stress factors.

$$\textbf{CF = 100 x Body weight (g) / (length (cm))}^3$$

➲ Observation using a light microscopy

Each sample was observed under microscopy connected to a performed computer. A software known as Cell Sens Dimension within the computer provided images for a further analysis and each image was captured using a digital camera. Five images are taken from each slide. Each image corresponded to a slide viewed using an objective lens of 20x magnification for cells structure overview and 40x magnification for a detailed cells structures. An immersion oil was used for 100x magnification which allowed an easy detection of organelles.

2.6. Image analysis

The image analysis consisted of comparing cells structures of liver and gills that were exposed to drill cuttings with those cells belonging to the control. The objective of this analysis was to identify any histopathological alterations in the tissues.

2.7.Score and statistical approach to data

Statistical analysis was performed using the Statistical Package JMP 11. Data were expressed by means ± standard deviation and coefficient of variation, Dunnett's test was used to determine differences between the control and exposed groups. The significance level was $P<0.005$. Null hypothesis was made between the control and the exposed groups where it was rejected if the test showed that p-value $p<0.05$.

3. RESULTS

3.1. Daily care parameter evaluation

Temperature, dissolved oxygen (DO) and flow rate is reported in the following paragraph as daily care parameters. Means ± standard deviations (SD) were calculated for both temperature, DO and flow rate.

➲ Temperature (T)

Temperature is a crucial environmental parameter in all studies with living organism. It is also one of the parameters needed to be carried out since it affects the solubility of the dissolved oxygen within the body of the water. When temperature increases the solubility of the DO decreases. Temperature data during the exposure experiment is plotted in figure 10.

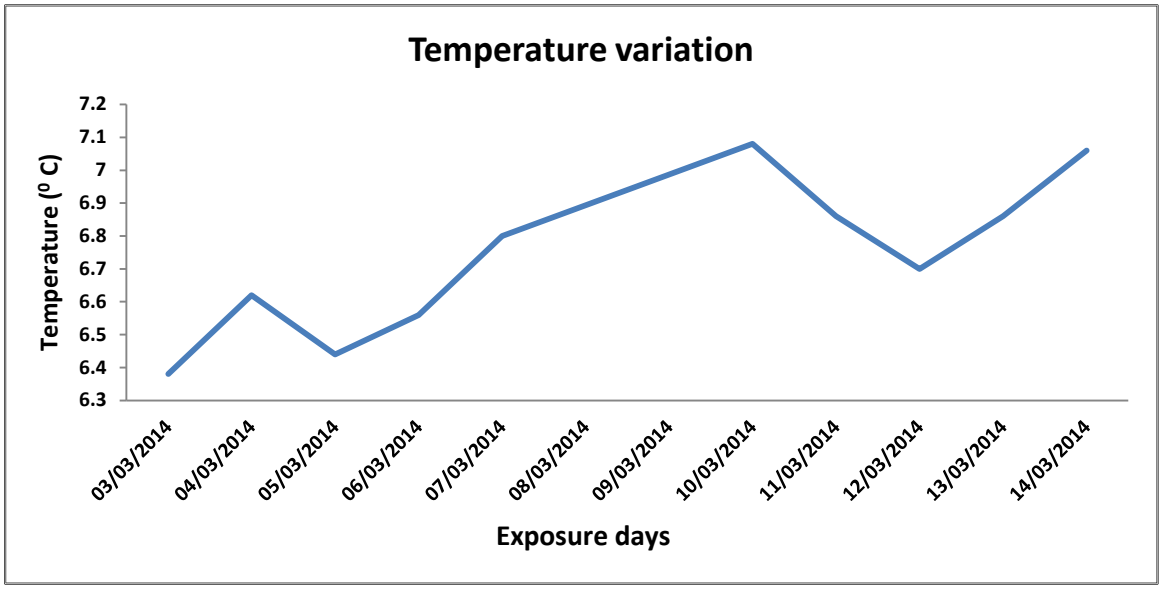


Figure 10: Temperature variation over the exposure time.

This curve shows the overall average temperature variation during the exposure period. No significant change of the temperature is recorded. Temperature varied between 6.4 ±0.2 oC to 7.1 ±0.2 oC. This range of temperature variation is suitable according to the life cycle history of *Salmo salar* in fresh water environment.

➲ Dissolved Oxygen (DO)

DO is an essential parameter in assessing water quality because of its influence on *Salmo salar* living with the body of the freshwater. The amount of DO in the body of water depends on the temperature variation. Figure 11 shows the DO fluctuation for the exposure days.

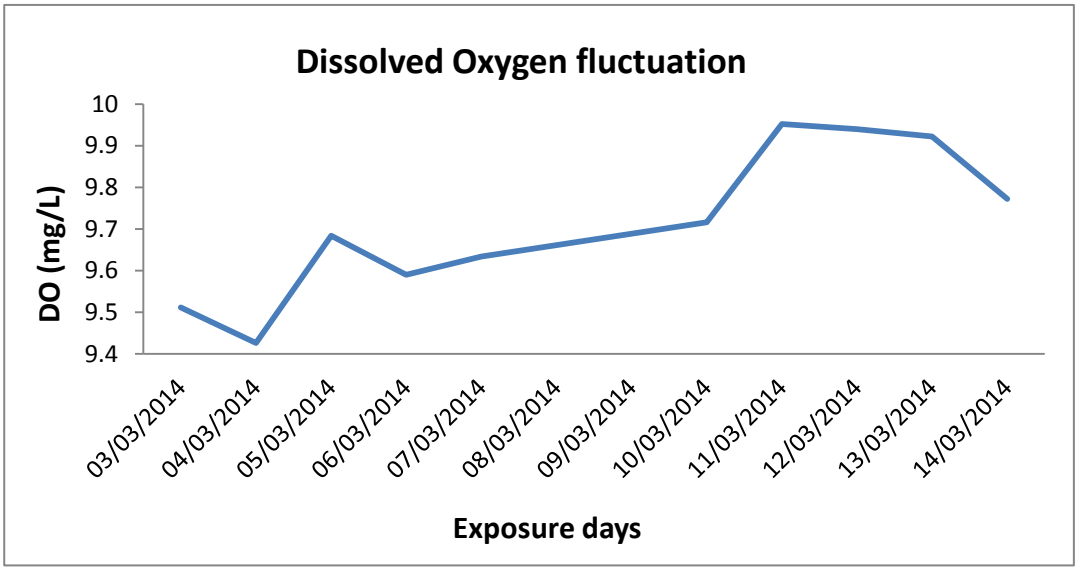


Figure 11: Dissolve oxygen variation over the exposure time.

From this graph, DO vary from 9.4 ±0.5 mg/L to 10.0 ± 0.1 mg/L which is suitable to keep Salmo salar in a good condition.

➲ Flow rate (Q)

Figure 12 shows the flow rate variation over the time of exposure. According to the graph, flow rate was kept in average range of 4.4 ± 0.4 L/min to 4.7 ±1.5 L/min. The change is not known to be significant even if it was challenging to keep the flow for all the tanks at the same amount. The continuous flow system gave an ideal oxygen supply, therefore no device aeration was used to supply the oxygen.

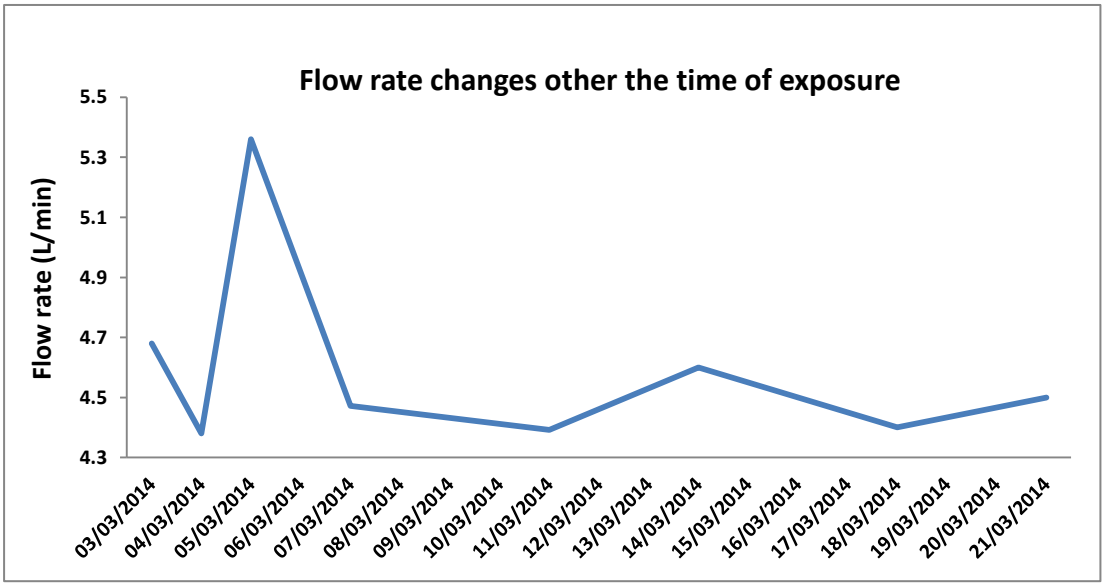


Figure 12: Flow rate variation over the time of exposure.

3.2. Support factors

Total organ weight (liver) and the surrounding parameters that influence the stability of the fish (CF) are reported as support factors. Results for both LSI and CF are compared with the control within the days of exposure to quantify the effect of the xenobiotics on Salmo salar. Raw data for both (LSI and CF) can be seen in appendix 4.

➲ Liver somatic index (LSI)

Figure 13 shows the effect of hydrophobic compounds adherent to the drill cuttings on the liver of Salmo salar.

From time zero (T0) to 3 days exposure, no observable changes could be noticed between the fourth groups (TDC LOW, UDC LOW, UDC HIGH, TDC HIGH) when referring to the control (figure 13a).

Seven days later, both low and high TDC remained at the same level as the control while both untreated surpassed the control by a significant difference. Seventy five percent of the livers are affected by the UDC LOW which was far above to the control. Down to 50%, the LSI data plot shows that effects persisted and it was not comparable to 75% of the control. UDC HIGH data index is lower than UDC LOW but still it shows significant changes to the fish liver (figure 13b).

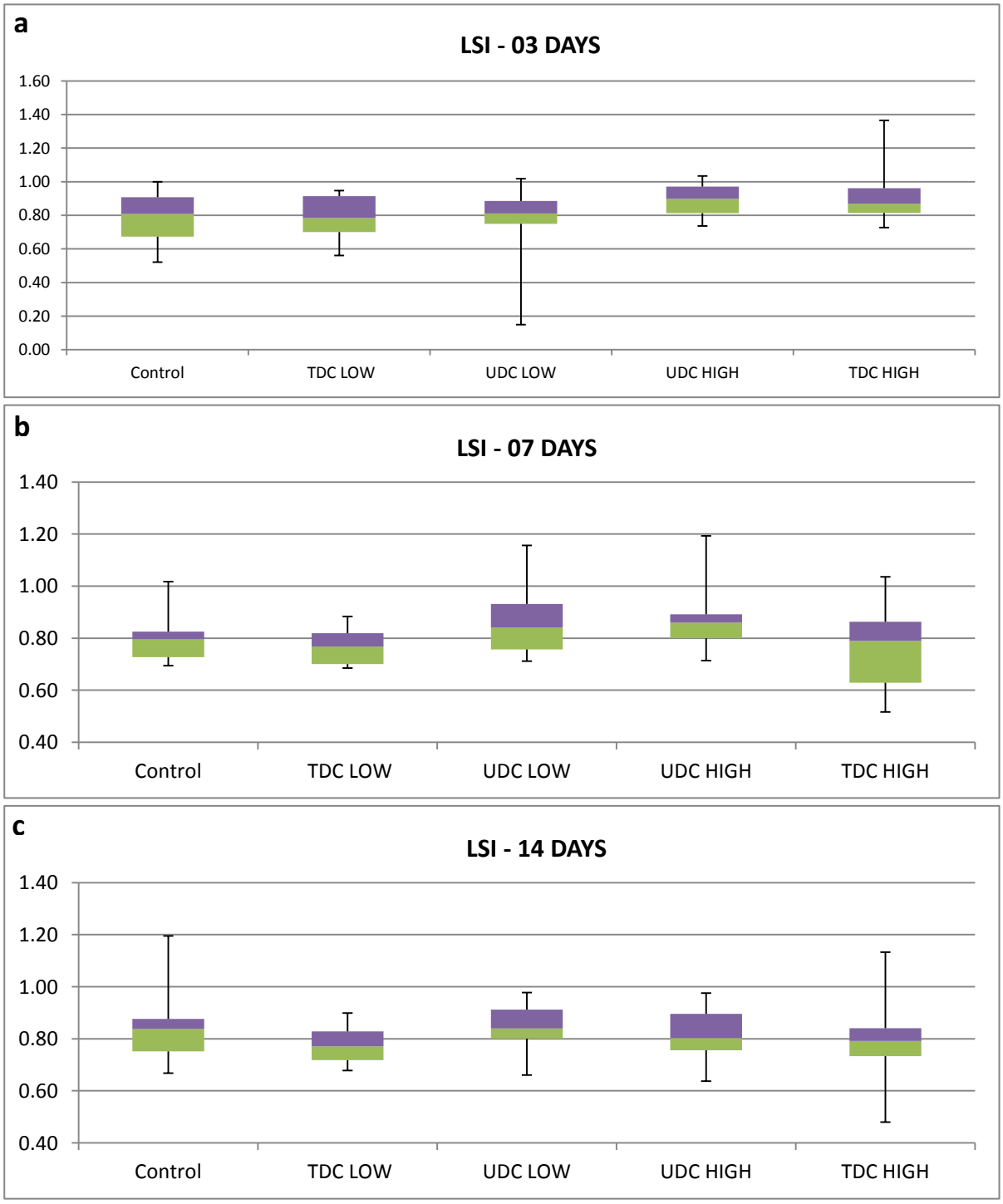


Figure 13.Liver Somatic Index (LSI) of *Salmo salar* after 3, 7 and 14 days of exposure to drill cuttings waste (oil based fluids). Median, 25%, 75%, minimum and maximum values are presented. Control=dechlorinated fresh water, TDC LOW= Low concentration of treated drill cuttings (0.1 ppm), UDC LOW= Low concentration of untreated drill cuttings (0.1 ppm), UDC HIGH= High concentration of untreated drill cuttings (1 ppm), TDC HIGH= High concentration of treated drill cuttings (1 ppm).

After 14 days, slight changes occurred between each exposure. The treated groups (low and high) decreased in a considerable range compared to the control by median, percentiles, max and min values but they remained in the same range as seen at 7 days. 75thpercentile both for treated group were quite lower compared to the median (control) while 75th percentile in both untreated are higher than seen in control and treated group (figure 13c).

➲ Condition Factor (CF)

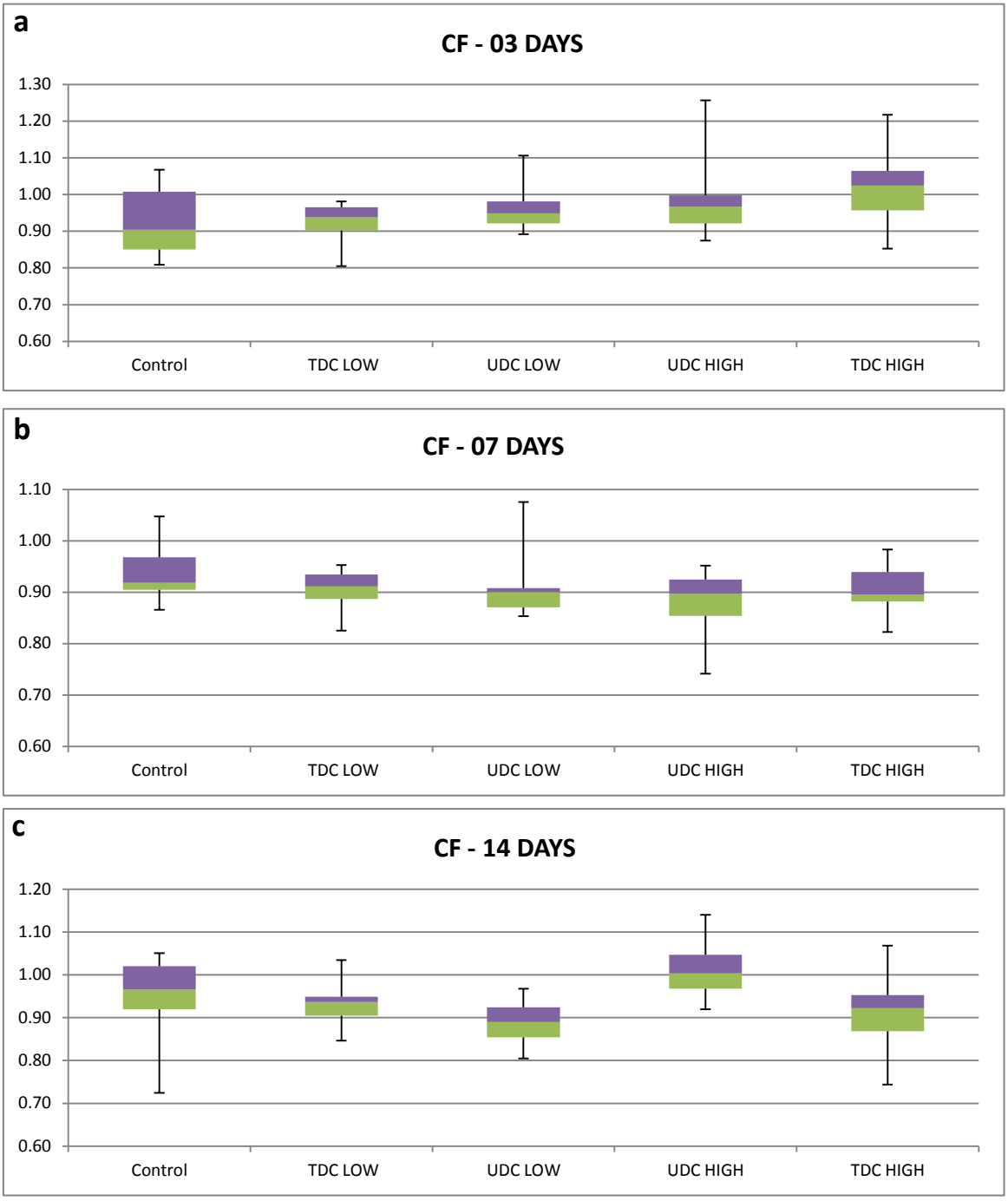


Figure 14.Condition factor (CF) of Salmo salar after 3, 7 and 14 days of exposure. Median, 25%, 75%, minimum and maximum values are presented. Control=dechlorinated fresh water, TDC LOW= Low concentration of treated drill cuttings (0.1 ppm), UDC LOW= Low concentration of untreated drill cuttings (0.1 ppm), UDC HIGH= High concentration of untreated drill cuttings (1 ppm), TDC HIGH= High concentration of treated drill cuttings (1 ppm).

No differences could be seen between groups (3 days of exposure).

After 7 days of exposure, fish were slightly stable in reference to the control (median and the 75th percentile for the control were higher than those seen for the treated and untreated group). Results showed as well that 75th percentile and the median tended to have similar value for untreated low (figure 14b).

After 14 days, no significant changes were seen between the exposed groups except the untreated high which percentiles, median, min and max were higher compared to the control (figure 14c) as well as the untreated high at 7 days (figure 14b).

3.3. Image analysis

- Gills histopathological alterations

Gills histopathological evaluations consisted of recording gills alterations by identifying alterations or damage resulting from exposure to drill cutting waste (oil based mud) using an atlas as reference. Photomicrographs of the gills are presented in the following section describing normal gills features; control and exposed gills arch.

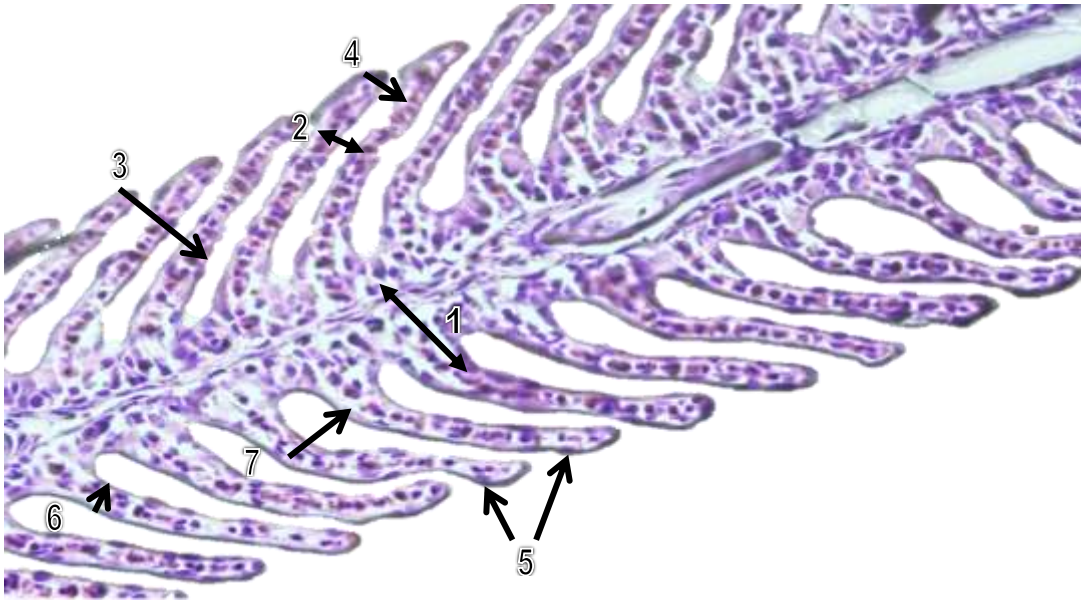


Figure 15: Photomicrograph of the gill of *Salmo salar* (Formalin, H&E, Bar = 6µm). Normal aspect of the gill showing: 1. Primary lamella; 2. Secondary lamella; 3. Pillar cell; 4. Mucuous cell; 5. Epithelial cell; 6. Chloride cell; 7. Lacuna (capillary lumen). Original magnification x 40.

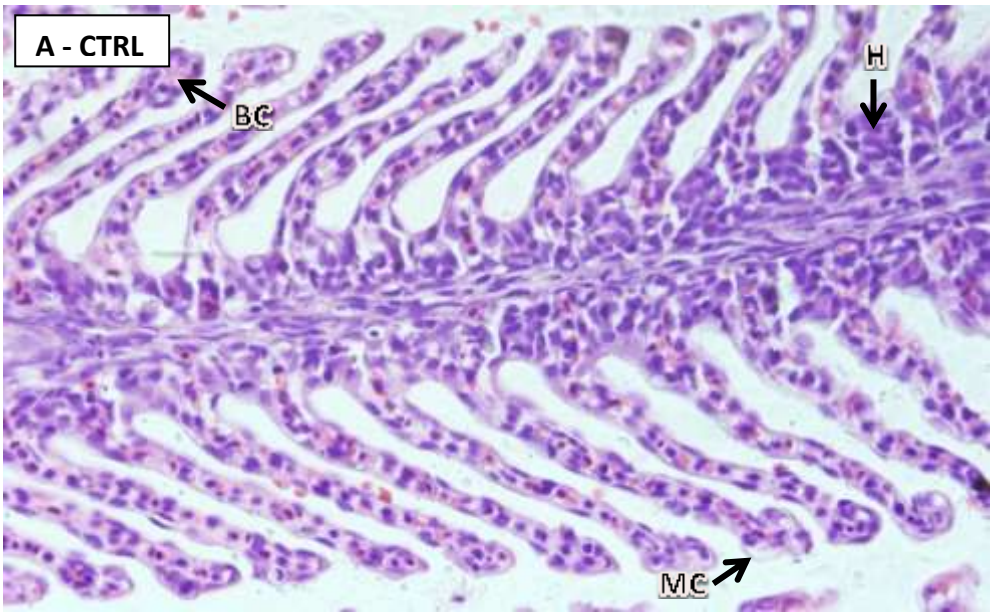


Figure 16: Gill tissue of *Salmo salar* (H&E, bar = 6µm, x40). (A) Control (non-exposed group). Hyperplasia (H), blood congestion (BC), mucous cell (MC).

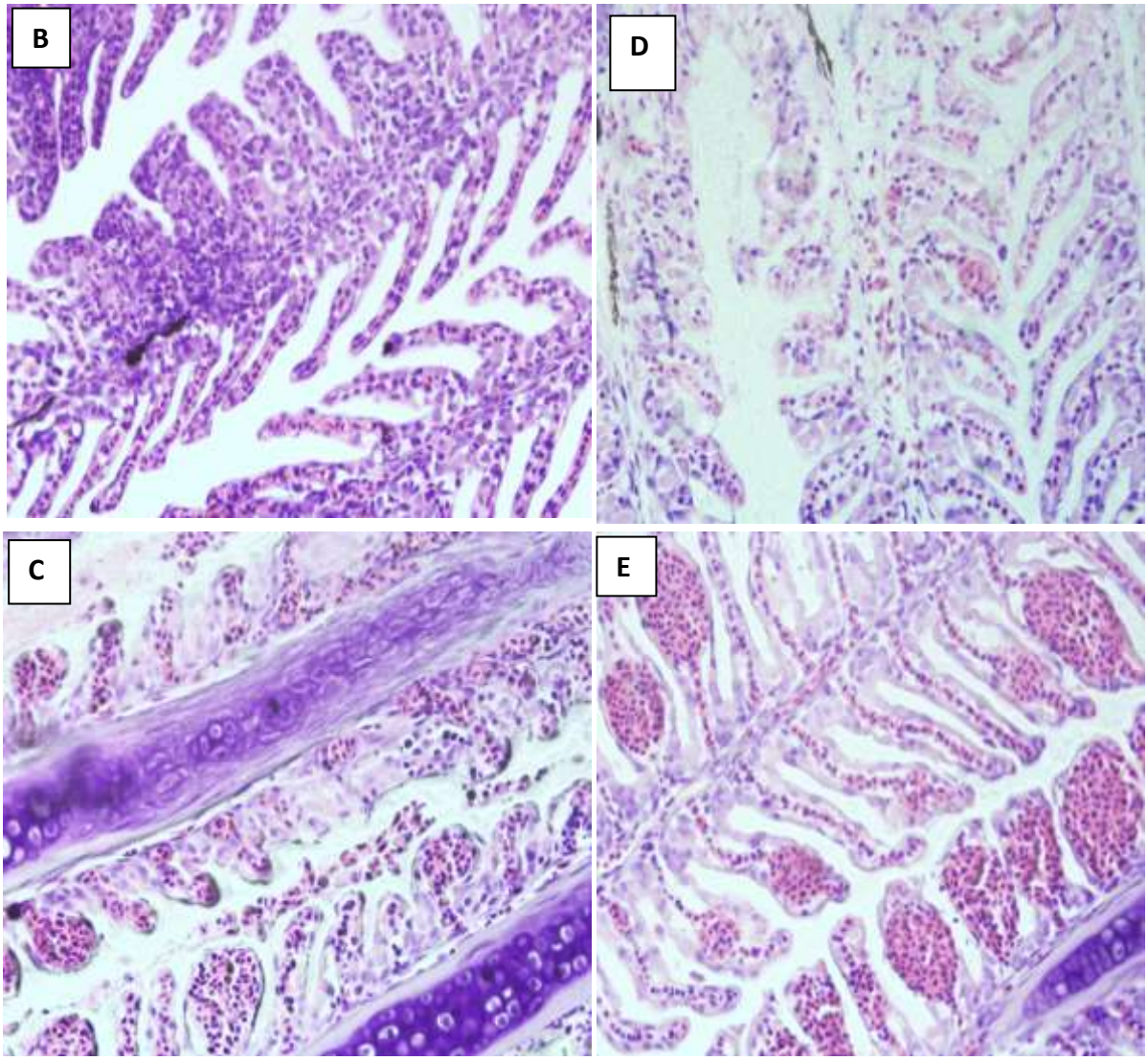


Figure 17: Histologic sections of gills of specimen of *Salmo salar* exposed to drill cuttings waste oil based mud (OBM). Formalin, H&E, bar = 6µm, x 40. (B) exposed to 0.1 ppm treated OBM showing hypertrophy of the secondary lamella, epithelial hyperplasia, blood congestion; (C) exposed to 1 ppm treated OBM with aneurism, epithelial hyperplasia, lamellar fusion, epithelial lifting, epithelial hypertrophy and necrosis; (D) exposed to 0.1 ppm untreated OBM affected by aneurism, epithelial lifting, epithelial hypertrophy, lamellar fusion, excess mucus secretion; (E) exposed to 1 ppm untreated OBM. Gill filament suffers of aneurism, epithelial lifting, excess mucus secretion, epithelial hypertrophy and necrosis.

➲ **Liver histopathological alterations**

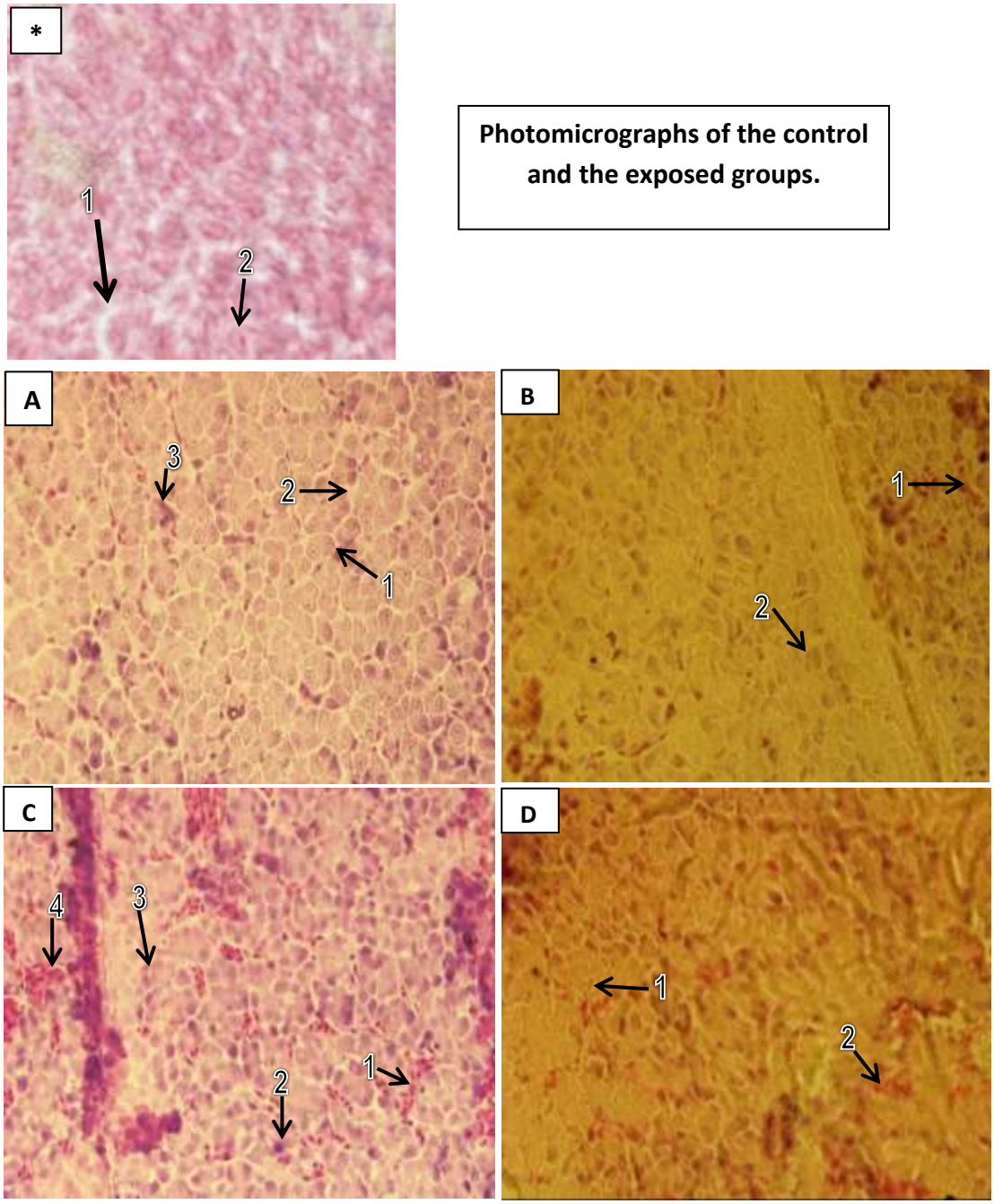


Figure 18: Liver tissue of Atlantic *Salmo salar*. Formalin, H&E, bar = 5µm, x 40. (A) Liver exposed to 1 ppm of treated OBM. 1- Swelling of hepatocytes. 2- Dilatation of sinusoid. 3- Blood congestion in the sinusoid. (B) Exposed to 0.1 ppm of treated OBM. 1- Blood congestion. 2- Swelling of hepatocytes. (C) Exposed to 1ppm of untreated OBM. 1- Blood congestion. 2- Nuclear hypertrophy. 3- Swelling of hepatocytes. 4- Massive infiltration of round cells (lymphocytes and macrophages). (D) Exposed to 0.1 ppm of untreated OBM. 1- Swelling of hepatocytes. 2- Blood congestion. (*) control. 1- Sinusoid. 2- Hepatocytes.

3.4. Score analysis

Median, mean, standard deviation (SD) and coefficient of variance (CV) were calculated using Microsoft Excel 2010. Score for gill alterations is presented in appendix 5. Table 6 below shows gills alterations data based on mean and SD.

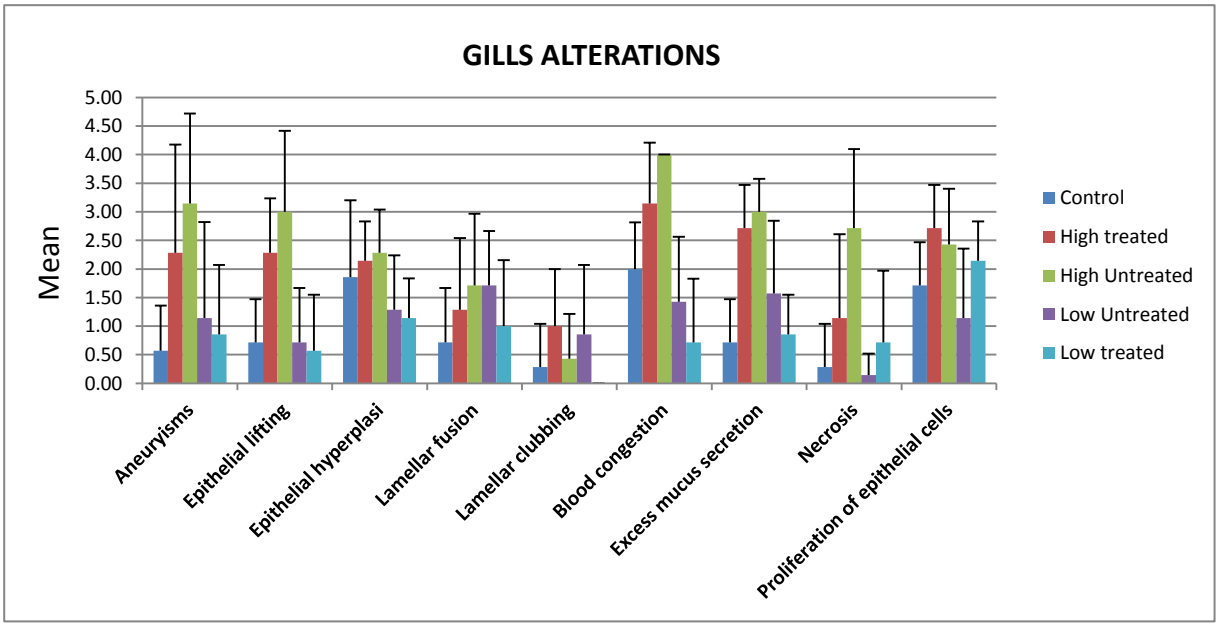


Figure 19: Gill histopathological responses to drill cuttings waste and control.

Figure 19 summarizes histopathological effects in the gill of Salmo salar exposed to oil based mud. Each alteration affected the gill whether in control or in the exposed groups. Mostly gill that were exposed to high untreated drill cuttings waste showed a higher effect compared to the control in each alteration except for the epithelial cells proliferation where gill exposed to high untreated had lower mean than the gill in high treated (control = 1.7 < high untreated = 2.4 < high treated = 2.7).

As will be discussed, some of these alterations were not significantly different between control and exposed groups. These were the cases of epithelial hyperplasia (EH), lamellar fusion (LF), blood congestion (BC), excess mucus secretion (EMS) and proliferation of epithelial cells (PEC). Lamellar clubbing affected the gill whether in control or in the exposed groups with less severity. Mean value showed differences between them (control, 0.71; low treated ≈ 1.29; high untreated equals to low untreated = 0.71).

The coefficient of variation showed that there was a small variation between control and exposed group in each of these alterations. Higher CV reflects more variation of the data

compared to the mean. As seen in table 6 (green column), the degree of variation presented by CV between control and exposed group in case of EH can be considered fairly low.

Table 6. Coefficient of variation of gills alterations within control and exposed groups.

	CV (%)								
	An	EL	EH	LF	LC	BC	EMS	Nec	PEC
C	138	106	72	133	265	41	106	265	44
HT	83	42	32	97	100	34	28	128	28
HU	50	47	33	73	184	0	19	51	40
LU	147	133	74	55	142	79	81	265	106
LT	142	171	60	115	0	156	81	175	32

Note: Arrows used inside of these red and green columns mean "between". (C) Control; (HT) high treated; (HU) high untreated; (LU) low untreated and (LT) low treated.

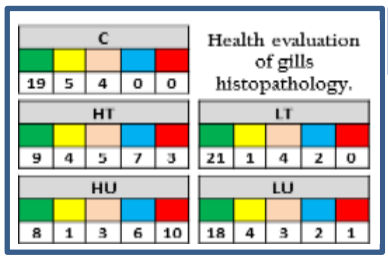
In opposite, data presented by aneuryisms (An), epithelial lifting (EL), lamellar clubbing (LC) and necrosis (Nec) showed a significant degree of variation as the CV had a greater percentage for each of these alterations. EL showed a greater variation: HT= 42 < HU= 47 < C= 106 < LU=133 < LT= 171 (Table 6, red column). Based on the CV, the following section showed the selected data that had a greater variation of the gill alterations.

Table 7. Gill histopathology scores and health evaluation colour codes

a

Sample ID	Treatment	An	EL	LC	Nec	
101	control	0	2	2	0	C
102	control	1	1	0	0	
103	control	0	0	0	0	
104	control	2	1	0	2	
105	control	0	0	0	0	
106	control	1	1	0	0	
107	control	0	0	0	0	

f



b

Sample ID	Treatment	An	EL	LC	Nec	
111	Tank 1	0	1	0	0	HT
112	Tank 1	1	3	2	0	
113	Tank 1	3	2	1	2	
114	Tank 1	4	3	2	3	
115	Tank 1	4	3	2	0	
116	Tank 1	4	3	0	3	
117	Tank 1	0	1	0	0	

d

Sample ID	Treatment	An	EL	LC	Nec	
141	Tank4	0	2	0	0	LT
142	Tank4	0	0	0	0	
143	Tank4	1	0	0	3	
144	Tank4	3	2	0	2	
145	Tank4	0	0	0	0	
146	Tank4	2	0	0	0	
147	Tank4	0	0	0	0	

c

Sample ID	Treatment	An	EL	LC	Nec	
131	Tank 3	0	0	1	0	LU
132	Tank 3	0	0	2	0	
133	Tank 3	0	0	0	1	
134	Tank 3	3	2	3	0	
135	Tank 3	1	0	0	0	
136	Tank 3	0	1	0	0	
137	Tank 3	4	2	0	0	

e

Sample ID	Treatment	An	EL	LC	Nec	
121	Tank 2	4	3	0	3	HU
122	Tank 2	4	4	1	4	
123	Tank 2	4	4	0	3	
124	Tank 2	4	3	0	4	
125	Tank 2	2	4	0	2	
126	Tank 2	4	3	0	3	
127	Tank 2	0	0	2	0	

The use of qualitative score data showed that gills for the control were more affected by epithelium lifting (more yellowish) than aneuryism and had a lower response to necrosis as well as lamellar clubbing (Table 7a). In case of high treated (HT), it showed a colourful texture but gill were basically affected by aneuryism (Table 7b). Aneuryisms were found in gills exposed to low untreated drill cuttings waste but it was in a very low effect. It has been scored with one red score (Table 7c). No red colour can be seen for gills exposed in the low treated (LT) group while green colour dominated the picture (Table 7d). Gills were mostly affected in the high untreated group. The red colour is well spread between aneuryism (5 red/7samples), epithelial lifting (3 red/7 samples) and necrosis (2 red/7 samples), but those gills were slightly free from lamellar clubbing (Table 7e).

When it comes to the general health of the gill, evaluation by colour is meaningful to distinguish the severity of gills damage within the exposed group compared to the control, summarized in table 7f. It has been seen that gills exposed in the high untreated group have more severe damage (10 reds) compared to control with zero red. Low treated group has no significant damage (zero red) and present a high healthy state (21 greens) which is more than the control by a difference of 2 greens.

Gills histopathological observations were grouped together and scored. As seen in figure 20, scores from 0 to 4 with its corresponding colour has been used to quantify the severity of each alteration within the control and exposed groups. By considering green (healthy) and red (damaged) as condition of health state, it turns out that gills can be set up chronologically as follows according to the qualitative data provided in table 7f:

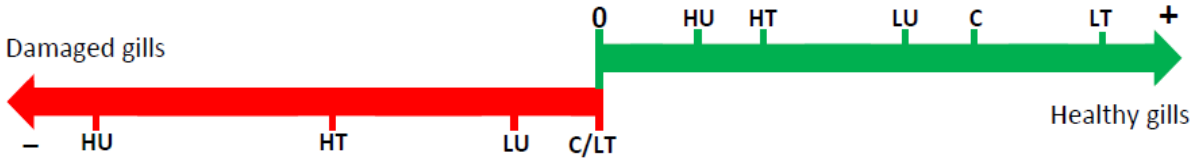


Figure 20: Gills chronological health evaluation.

Tests of statistical differences have been performed using the subjective scoring data. Being subjective score values these tests do not formally yield results of statistical significance. However, it is still instructive to discriminate the histopathological changes that can be considered as high and low, and it is therefore done and presented in the following.

➲ Gill histopathology

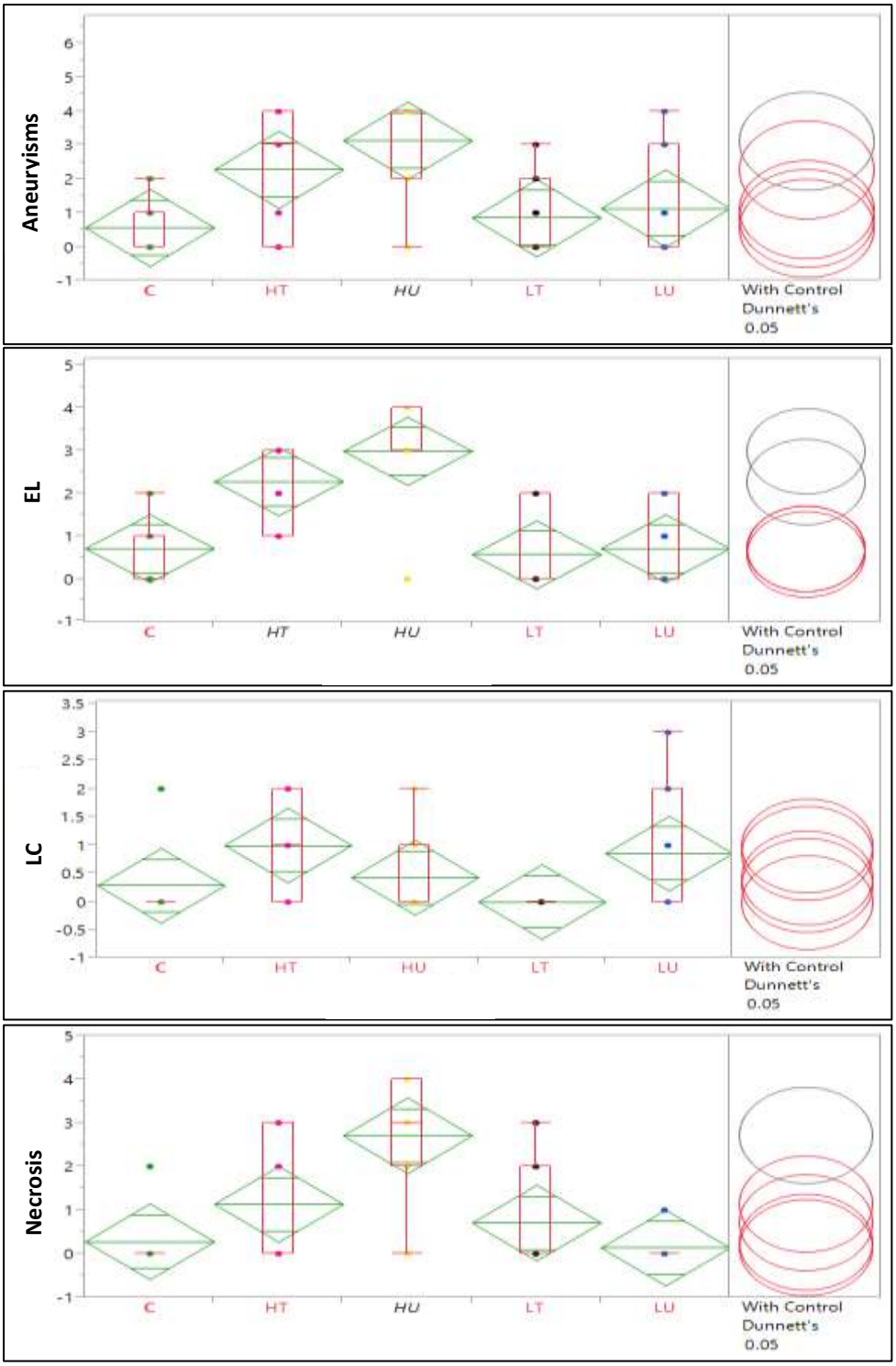


Figure 21: Dunnett's test on gills alterations in the exposed group compared to the control group.

"Significant" differences can be shown by the tendency of the Dunnett's circle that moved away from the control and black test of the different groups. See text for explanation of "significant" differences.

According to Dunnett's test, gills exposed to high untreated were more affected by aneuryisms compared to the control. The control has a p-value = 1 while the high untreated comes out with 0.01 which applied to objective data would have been significantly different (figure 21A). Such findings are denoted as "significant" in the following.

The EL graph (figure 21B) showed "significant" changes in both the high untreated and high treated groups. Their p-value were respectively 0.001 (HU) and 0.03 (HT) (both $p<0.05$). It was also interesting to note that these discriminates HU and HT were in the expected way.

No significant differences were found for the LC graph as all the p-values were above 0.05 (figure 21C).

Necrosis showed a "significant" effect in gills exposed to high untreated ($p= 0.001$).

4. DISCUSSIONS

4.1. Support factors

➲ Liver somatic index (LSI)

Liver somatic index, a sensitive parameter, is one of the indices that gives a good understanding of the health of the fish when environmental changes occur. In accordance to the exposure that was taken for 2 weeks, no periodic variation was seen at 3 and 7 days of exposure while after 14 days, a slight changes occurred for fish exposed to untreated group. This can be explained as a result from the uptake of some lipophilic compounds from the drill cuttings that were stored in the liver, therefore lead to the change of the liver weight. The increased volume of the liver was not seen in 3 and 7 days possibly because of time dependence. It has been stated by Hoque et al., (1997), that usually there is no significant change in LSI within a short time of exposure.

➲ Condition factor (CF)

Condition factor is a coefficient that describes mostly the individual growth of the fish. In this present study, no significant changes were seen for any of the fish exposed to drill cuttings waste, nor the control. Three, 7 and 14 days of exposure were carried out, none of them shows any noticeable variation when compared to each other. It is believed that this result is related to the relatively short exposure time (5 weeks including the acclimation days). Hence short time exposure duration does not reveal any proper Fulton's condition factor (Julie et al., 2004).

4.2. Image analysis

➲ Gills histopathology

Fish that inhabit polluted environment are particularly susceptible to contaminants (soluble and suspended) that can damage gill structure and physiology. Metals and relatively low-molecular weight organic compounds are readily absorbed across the gill (Randall et al., 1996). These contaminants cause deleterious changes in cellular structures, including the epithelium and pillar cells (Al-Attar, 2007). The present study showed that gills exposed to treated and untreated drill cuttings waste oil based muds within 14 days presented a higher occurrence of histopathological lesions as shown in figure 17. The severity of the implications in gill tissue varies in accordance to the type of the exposed group but it can be noted that epithelial lifting is common for gill histopathology both in low and high concentration of the

exposed groups. Health (1995) stated that lifting of the lamellar epithelium is one of the first changes in fish gills under acute exposure to toxic substances such as oils and metals. This statement supports the result of this study, where the chemical analysis of the body of water in which fish were exposed showed both presence of metals (lead, cadmium, nickel, copper) and PAHs (appendix 6), these may have affected the fish gills. As consequences of the epithelial lifting, an increased diffusion distance between water and blood occurs, impairing oxygen uptake. As consequence, fish increase their rate of respiration by compensating for the low uptake of oxygen (Fernandes and Mazon, 2003). This is one factor contributing to make fish gills a sensitive organ that is easily damaged by numerous pollutants, even at low concentrations (Karlsson, 1983) and thus an interesting biomarker for monitoring such effects. PAHs have been reported to cause structural damage to the respiratory lamella of the gills (DiMichele & Taylor 1978, Correa & Garcia 1990, Prasad 1991, Nero et al. 2006; Santos et al., 2011). This referred especially to naphthalene due to its high acute toxicity and low molecular weight (Vijayavel et al. 2004). In this project, PAH metabolites were measured in bile at 14 days and revealed the presence of pyrene and naphthalene at significant levels (Sanni et al. 2014). Therefore, damages in fish gills that the salmon encountered in this study could have been mainly caused by the severe action of naphthalene since those actual damages are the most common alterations in the fish exposed to it (Santos et al., 2011). These damages that change gills structure include aneurisms, necrosis, epithelial hyperplasia with lamellar fusion, epithelial hypertrophy, and epithelial lifting. Similar gills alterations were stated by other authors such as Baker, (1969); Gardner and Yevich, (1970); Van der Putte and Paert, (1982); Hemalatha and Banerjee, (1997) and Au, (2004) reporting that these modifications are typical histopathological lesions of gills in response to a wide range of contaminants such as petroleum compounds and heavy metals. The change of gill structure is then the response to toxicants exposure (Mallatt, 1985; Wood, 2001; Au, 2004). Metal accumulation in gills is believed to be part of lamellar modifications because of their external location and the close contact with the water that contains toxic compounds which allow them to be absorbed through the delicate epithelium. Hence, the lamella epithelium lining reacts to the toxic compounds leading tissue damage related osmoregulatory imbalance. Thus, modifications to the physiological property and morphological shapes that are evident changes observed at the lamella and which is best explained by intake of those metals mostly occurs via the gills.

➲ Liver histopathology

Liver samples that are analyzed by the use of a liver histopathology atlas showed different types of alterations within the sample. The severity of the damage that livers encountered varies in accordance to the state of the drill cuttings waste (treated and untreated) as well as the concentration that has been chosen for the exposure (low and high).

After 14 days of exposure, liver tissue of Salmo salar was affected highly by damages such as swelling of hepatocyte, massive infiltration of round cells, blood congestion in the sinusoids, nuclear hypertrophy and dilatation of the sinusoid. These histopathology if not handled lead to the malfunction of the liver. It is believed that these modifications in the liver tissues are caused by toxic xenobiotic compounds that normally do not have any affinity with the liver tissues, and hence the tissues do not recognize them and react to them. Thus, it is evident that lesions occur because of the response of the liver tissues against the noncellular xenobiotic compounds which are the polycyclic aromatic hydrocarbons known as PAHs.

PAHs are widespread contaminants and known to have a carcinogenic and mutagenic character (Yan, 1985; White, 1986). It is a hydrophobic organic chemical compound that is likely seen in drill cuttings (Okparanma et al. 2010) hence they tend to rapidly adsorb on particles (Neff, 1979). As PAHs are lipophilic it is the reason to believe that when fish were exposed to drill cuttings oil based mud then PAHs were taken up by the exposed organisms and accumulated in the liver. A study conducted by Gonzalex et al. (1993) and Bernet et al. (1999) stated that liver comes into direct contact with pollutants absorbed from the environment, therefore lesions in the liver are associated with contaminants existing in the body of the freshwater. PAHs that have reached the fish liver will accumulate there. Liver is known to be a multifunction organ for purposes such as storage (lipids, carbohydrates), detoxification (pollutants, toxins) and immune defense (Brusle and Anadon, 1996). This explains presence of PAHs in the liver of the salmon in this study, but even though the pathway of PAHs has its end into the liver it is not sufficient to conclude that these livers histopathology alterations are resulted from uptake of PAHs. The image analysis does not provide such information of xenobiotic specificity. Therefore, other toxicants such as heavy metals could alternatively be the main cause of these lesions.

When the exposure was carried out several biomarkers were studied in the same study as the liver and gill histopathology. These biomarkers include nuclear aberrations, glutathione S-transferase (GST), ethoxyresorufin O-deethylase (EROD), catalase (oxidative stress biomarker), lysosome membrane stability (LMS) and PAH metabolites.

PAH metabolites were measured in bile using fixed wavelength fluorescence. This biomarker is important for understanding the liver histopathology since it measured the PAHs content in the bile of the fish as bile receives excretion from the liver. In other words, determination of PAH metabolites in bile serve as a tool for assessing environmental PAH exposure in fish (Beyer et al., 2010). Hence, result from the PAH metabolite in bile reported the presence of naphthalene, pyrene and benzo(a)pyrene but only the pyrene and naphthalene were in any considerable amounts after 14 days of exposure (Sanni et al., 2014). The PAH metabolite result supports the argument that fish were exposed, had taken up and had accumulated PAHs. Hence, the bioaccumulation of these hydrophobic compounds might have reached levels in which liver function might be impeded, thus resulting in possible gradual degeneration of the liver cells.

According to the figure 18, the degree of severity of the liver damage is not the same between the exposed groups. Liver exposed in untreated group (figure 18C) is more damaged than those exposed in treated group (figure 18A). The reason can be thought mainly as the result of the thermo-mechanical treatment since this method reduces the toxicity level of PAHs in the treated drill cuttings waste but might increase the bioavailability of the heavy metals (Randrianarimanana, 2014). Heavy metals react as well in liver cells.

An earlier study, liver exposed to lead at high concentration (0.006 mg/L) showed deformities such as dilatation of the sinusoids within 9 days after exposure (Olojo et al. 2005). This kind of alterations was seen in the liver of Salmo salar. The treated drill cuttings contain heavy metal like lead but in a very low concentration equal to 0.0003mg/L. As liver is a very sensitive organ then it could be believed that the accumulation of lead in the liver can be the reason of such lesions (dilatation of sinusoids).

4.3. Score analysis

- **Liver histopathology**

It was attempted to provide score data for liver histopathology alterations in this study but for technical raisons most of the liver samples were destroyed prior to the staining with haematoxylin and eosin. Preparations of new samples were not possible because of the short timeframe of this thesis. According to the performed liver image analysis, some notable changes occurred. It is believed that also liver histopathology could give interesting data for evaluation of high order effects in the context of drilling waste exposure.

➲ Gill histopathology

According to the score results, significant differences between exposed groups in comparison to the control were clearly seen. In order to test these difference more thoroughly a semi-statistical "significance" testing was done on the means of the subjective score values.

Null hypothesis:

None of the alterations that may affect the gill, in the exposed groups were different.

1. For aneuryisms, the test showed that the high untreated was "significantly" different from the control (P=0.0004). Thus, the null hypothesis is therefore rejected at a chance of 0.04% of being wrong.

2. For epithelial lifting, P was 0.0001 for the high treated and high untreated which means that the null hypothesis is also rejected for these lesions. This can be concluded with 0.01% chance to be wrong.

3. For lamellar clubbing, P=0.06 for all the exposed groups which is higher than the confidence interval 0.05, meaning that the null hypothesis is not rejected and the capacity of the exposed groups to induce lamellar clubbing are not "significantly" different.

4. In case of necrosis, P=0.0001 for high untreated which result is the same as seen for epithelial lifting. Case and the null hypothesis is rejected with 0.01% of being wrong.

This testing confirmed the indications of elevated aneuryisms, epithelial lifting and necrosis, while it did not confirm the same for lamellar clubbing. These statistical data collected from the gills histopathology are very subjective since the scoring system depends on self-perception even though gills histopathology atlas was used. Thus, it makes statistical analysis difficult to carry out. Nevertheless, based on the scoring data used it is of particular interest to note that Dunnett's test was useful for analysing gills histopathology data.

Other studies (Paulo et al., 2012; Al-Attar, 2007) have used ANOVA (one-way analysis of variance) with the disadvantage that ANOVA does not express which means differed. Thus, the analysis always requires a post-hoc test or Student's t-test or Tukey test while Dunnett's test not only gives an easier way to explain data but also provides the "semiquantitative significance" in one step. This might be an interesting way to analyse these types of data in the future if the histopathological assessments will yield more objective quantitative data.

5. CONCLUSION AND FUTURE PROSPECTS

Untreated oil based mud is not allowed discharged or disposed today due to the environmental hazard associated. The results of this study may imply that the oil based drilling waste with thermo-mechanical treatment can still represent a threat to environmental quality due to the high presence of severe pathological alterations in the liver and gills of salmon parr (Salmo salar). Furthermore, these results combined with data supplied by other studies indicate that gills and liver alteration agents such as heavy metals and PAHs are affecting the salmon. PAHs hold a great environmental concern due to their toxicity and persistence in marine environments (Lee & Neff, 2011), especially those 16 compounds that are defined by EPA including bezo(a)pyrene, pyrene and naphthalene. Thus, it is imperative that mitigation measures such as improving the efficiency of the TTC method is taken to ensure that no damage will occur when treated oil based drilling waste is in contact with the environment.

Biomarkers have been used in environmental monitoring to assess the effect of pollutants on the environment. They exist in a wide range from molecular level to organism via tissues and organs. Their importance depend on their capacity of being sensitive to a particular stressor which makes them useful as indicators of both exposure and effects (Van der Oost et al., 2003). Besides biochemical biomarkers, physiological and morphological parameters are higher-level responses following chemical and cellular interaction, which are generally indicative of irreversible damage (Hinton et al, 1992). In accordance to this study, high order of biomarkers were focused on gill and liver histopathology. Exposed to PAHs and heavy metal components of the drilling waste for 14 days, gills of Salmo salar revealed high tissues modifications and observations of behavioural change of the fish trying to stabilise its physiological systems. These gill modifications were seen in 7 individual of salmons among ten samples from each of the exposed groups. The same severity of damages was seen in the salmon livers from the exposed groups, but damages were not scored since that many of the liver samples were destroyed. As opposed to the exposed groups, both gills and liver histopathology from the control were not significantly harmed, and it is believed that some few observed lesions are related to their confinement in the unnatural experimental habitat. Thus, a conclusion of the present study is that histopathological biomarkers can be valuable indicators of impaired health of fish and can reflect the effects of exposure to untreated and treated drilling wastes.

While gills and liver histopathology responded well to the drilling wastes stressor components (PAHs and heavy metals) and being a high order biomarker of effect, they also showed more sensitive responses than biomarkers at lower level observed both in the present study and in other recent studies with crude oil exposure (Sanni, S. *pers. comm.*). The sensitivity of these biomarkers was so high and relatively immediate that they may even be considered as suitable to serve as biomarkers of exposure to drill cutting discharges along with PAH metabolites. The objective in the present study was to evaluated toxicity and toxicity biomarkers in response to drilling discharges in fresh water. The issue is of equal importance in offshore drilling activities and it is based on the findings here recommended to launch the same experiment again but using marine organisms such as:

- A filter feeder (marine bivalve) which will show the degree of the bioavailability uptake and effects of the toxics compounds once settled down on the sediment.
- Pelagic fish (e.g. cod) that will provide information about the hazards possibly associated with spreading of the xenobiotic compounds in the seawater column.

These will provide useful data for evaluation of environmental risk in the marine pelagic and benthic environmental and they can possible serve as parameters for future biomonitoring to safe guard the marine environment if possible allowance to discharge thermo-mechanically treated drill cuttings to the sea is given.

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APPENDICES

Appendix 1

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Vår ref: 2014-01386
Utgave 1
Dato: 06.mar.2014

Deres ref: PR-10305

Laboratorierapport

Hensikt:	Analyse av TPH, PAH og metaller i faststoff.
Prøvested:	n/a
Prøve tatt av:	Universitetet i Stavanger
Mottatt dato:	19.feb.2014
Analysert:	20.feb.2014

Ved spørsmål angående denne rapporten, ta kontakt med undertegnede.

Med hilsen
Intertek West Lab AS

Teknisk ansvarlig

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Analysert av:

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Laborant
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i

Laboratorierapport

Prøveinformasjon

Prøve nr	Prøvepunkt	Prøve tatt dato
2014-01386	n/a	
-001	Prøve 1	
2014-01386	n/a	
-002	Prøve 2	

Resultater

Komponent	Enhet	001	002
Olje i sand	mg/kg TS	160000	960
Olje i sand (wt%)	wt% TS	16	0,096
* Tørrstoff innhold	wt%	66,0	84,6
Kvikksølv i faststoff, FIMS	mg/kg TS	0,37	0,049
* Naftalen	mg/kg TS	5,0	0,043
* Acenaftylen	mg/kg TS	1,7	<0,05
* Acenaftene	mg/kg TS	3,3	<0,01
* Fluoren	mg/kg TS	2,0	0,038
* Fenantren	mg/kg TS	2,1	0,13
* Antrasen	mg/kg TS	0,37	0,014
* Fluoranten	mg/kg TS	0,26	0,021
* Pyren	mg/kg TS	1,2	0,061
* Benzo(a)antrasen	mg/kg TS	0,26	0,028
* Krysen	mg/kg TS	0,30	0,046
* Benzo(b)fluoranten	mg/kg TS	0,15	0,041
* Benzo(k)fluoranten	mg/kg TS	0,017	<0,01
* Benzo(a)pyren	mg/kg TS	0,12	0,031
* Indeno(1,2,3-c,d)pyren	mg/kg TS	0,037	0,022
* Dibenz(a,h)antrasen	mg/kg TS	0,031	0,015
* Benzo(g,h,i)perylen	mg/kg TS	0,16	0,098
* Sum 16 EPA-PAH	mg/kg TS	17	0,59
* Kadmium, Cd	mg/kg TS	0,22	0,35
* Krom, Cr	mg/kg TS	22	26
* Kobber, Cu	mg/kg TS	74	78
* Nikkel, Ni	mg/kg TS	22	36
* Bly, Pb	mg/kg TS	64	70
* Sink, Zn	mg/kg TS	100	120

Tegnforklaring: * = Ikke akkreditert analyse

(n) = Antall replikater rapportert hvor n er replikat nummer.

Kommentarer

Prøve nr.	Prøve kommentar
-001	Prøve oppsluttet med Aqua Regia før analyse av Cd, Cr, Cu, Ni, Pb og Zn på ICP. Metodereferanse NS4770 gjelder kun for analyse av kvikksølv.
-002	Prøve oppsluttet med Aqua Regia før analyse av Cd, Cr, Cu, Ni, Pb og Zn på ICP. Metodereferanse NS4770 gjelder kun for analyse av kvikksølv.

Laboratorierapport

Metode referanse

	Parameter	Enhet	PKG Nedre	PKG Øvre	Metode	Standard	Usikkerhet
Kvikksølv i faststoff, FIMS							
	Kvikksølv i faststoff, FIMS	mg/kg TS	0,01		M-020	Mod. NS-EN 1483	±30% / ±0,01
Tungmetaller i faststoff, ICP							
*	Kadmium, Cd	mg/kg TS	0,08	2500		NS 4770/ICP-OES	±15% / ±0,08
*	Krom, Cr	mg/kg TS	0,08	5000		NS 4770/ICP-OES	±20% / ±0,08
*	Kobber, Cu	mg/kg TS	0,12	2500		NS 4770/ICP-OES	±20% / ±0,12
*	Nikkel, Ni	mg/kg TS	0,08	10000		NS 4770/ICP-OES	±20% / ±0,08
*	Bly, Pb	mg/kg TS	0,4	20000		NS 4770/ICP-OES	±30% / ±0,8
*	Sink, Zn	mg/kg TS	0,08	20000		NS 4770/ICP-OES	±20% / ±0,2
Olje i sand, GC/FID							
	Olje i sand	mg/kg TS			M-040		±20% / ±20
PAH_NPD_W							
*	Naftalen	mg/kg TS	0,02			ISO 18287	±30% / ±0,04
*	Acenaftylen	mg/kg TS	0,05			ISO 18287	±50% / ±0,1
*	Acenaftene	mg/kg TS	0,01			ISO 18287	±30% / ±0,02
*	Fluoren	mg/kg TS	0,01			ISO 18287	±30% / ±0,02
*	Fenantren	mg/kg TS	0,01			ISO 18287	±30% / ±0,02
*	Antrasen	mg/kg TS	0,01			ISO 18287	±30% / ±0,02
*	Fluoranten	mg/kg TS	0,01			ISO 18287	±30% / ±0,02
*	Pyren	mg/kg TS	0,01			ISO 18287	±30% / ±0,02
*	Benzo(a)antrasen	mg/kg TS	0,01			ISO 18287	±30% / ±0,02
*	Krysen	mg/kg TS	0,01			ISO 18287	±30% / ±0,02
*	Benzo(b)fluoranten	mg/kg TS	0,01			ISO 18287	±30% / ±0,02
*	Benzo(k)fluoranten	mg/kg TS	0,01			ISO 18287	±30% / ±0,02
*	Benzo(a)pyren	mg/kg TS	0,01			ISO 18287	±30% / ±0,02
*	Indeno(1,2,3-c,d)pyren	mg/kg TS	0,02			ISO 18287	±30% / ±0,02
*	Dibenz(a,h)antrasen	mg/kg TS	0,01			ISO 18287	±30% / ±0,02
*	Benzo(g,h,i)perylen	mg/kg TS	0,01			ISO 18287	±30% / ±0,02
Tørrstoff og gløderest i slam/faststoff, gravimetrisk							
*	Tørrstoff innhold	wt%	0,8	100	X-08-1	NS 4764	±10% / ±0,8

Tegnforklaring: * = Ikke akkrediterte analyser, PKG = Praktisk kvantifiseringsgrense. # = Analysen er utført av underleverandør.

Usikkerheten er angitt med 95% konfidensintervall. Der det er oppgitt både relativ og absolutt usikkerhet gjelder det argumentet som til enhver tid representerer størst usikkerhet.

Hematoxylin – Eosin
Rutinefarging

Fiksering: formalin; flere andrefiksativer kan benyttes

1. Snittene føres til vann
2. Farges 4 minutter i hematoxylin
3. Min. 10 minutter i vann for blåning
4. Farges 2 minutt i esosin
5. Skilles i vann (forholdsvis raskt)
6. Alkoholrekken til xylene, monteres

«Mayer's» hematoxylinløsning:

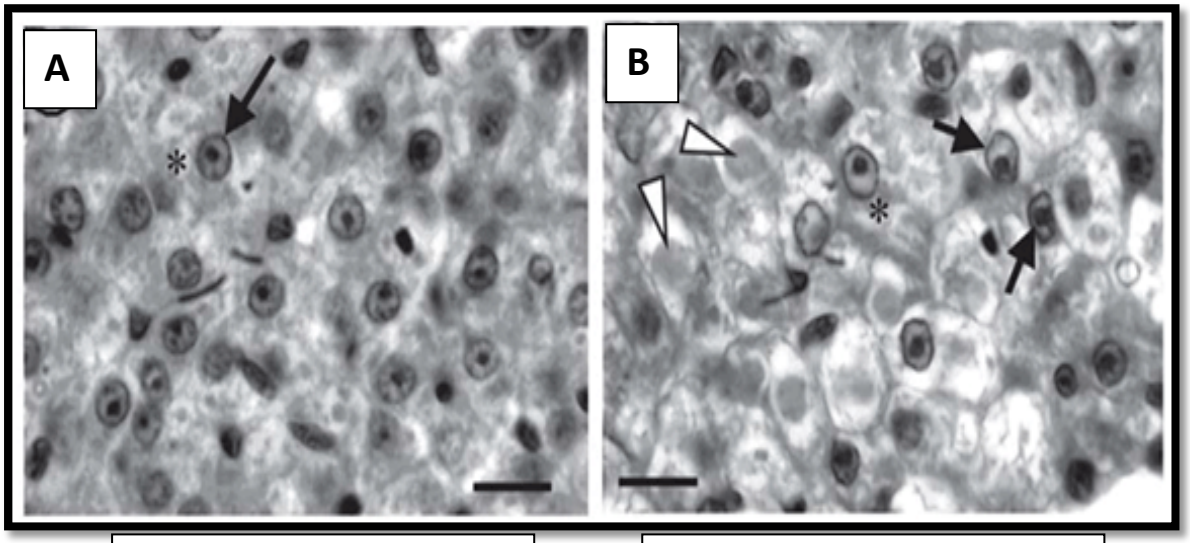
2000ml destillert vann
100g kaliumaluminiumsulfat, kalialun (Kal(SO4)2); rør godt. 4g hematoxylin tilsettes,
0.4g natriumjodat (NalO3) tilsettes. Filtreres før bruk.

Bruksløsning:
200ml hematoxylinløsning tilsettes 1ml 10% eddiksyre
Bruksløsning av eosin:
50ml 2% eosinløsning (Eosin Y) i 150ml des.vann tilsettes 1ml 10% eddiksyre.

Resultat:
kjerner, RNA rikt cytoplasma, calsium ..... blå
Muskulatur, fibrin, keratin ..... rødt
Kollagen ..... rosa
Erythrocyter ..... rødorange

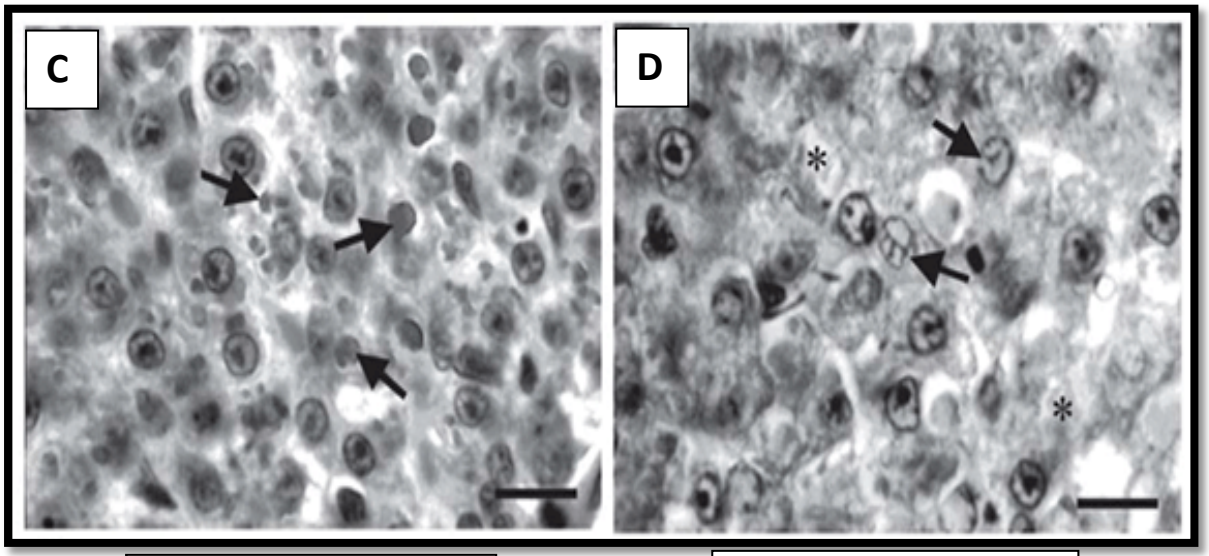
Modifisert fra:
«Cellular Pathology Technique»
Culling, Allison, Barr, 4.utgave, 1985, side: 157,160

LIVER HISTOPATHOLOGY ATLAS



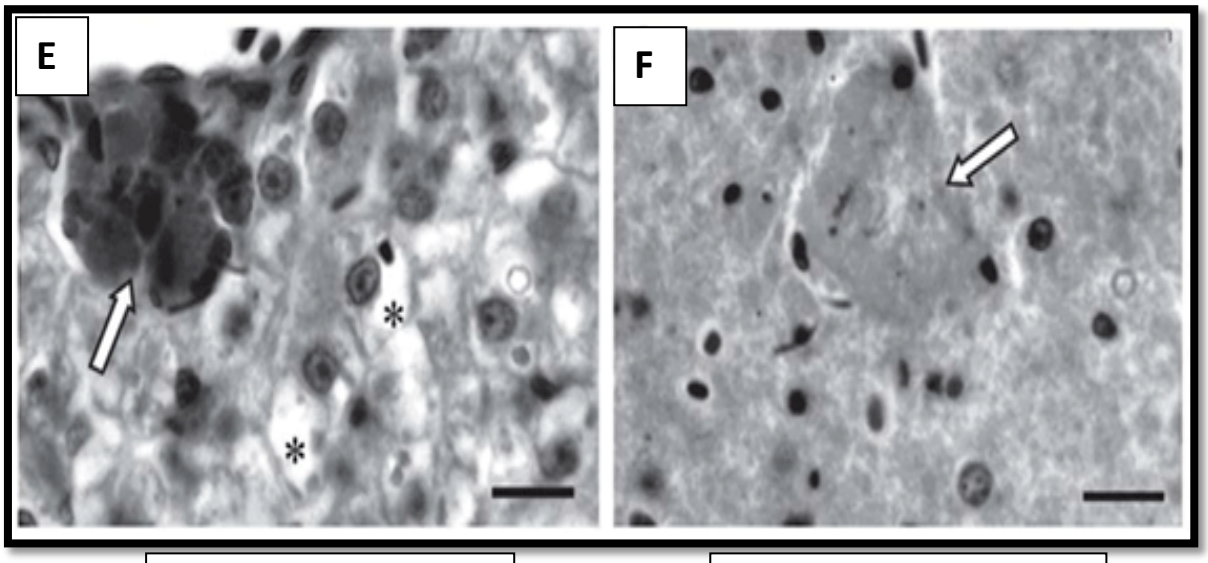
A. Normal hepatic tissue showing hepatocytes with granular cytoplasm (*) and central and round nucleus (arrow). Scale bar 10mm, H&E.

B. Hepatocytes with irregular shaped nucleus (black arrows), eosinophilic granules in the cytoplasm (arrowheads) and nuclear hypertrophy (*).Scale bar 10mm, H&E.



C. Bile stagnation (arrows). Scale bar 10mm, H&E.

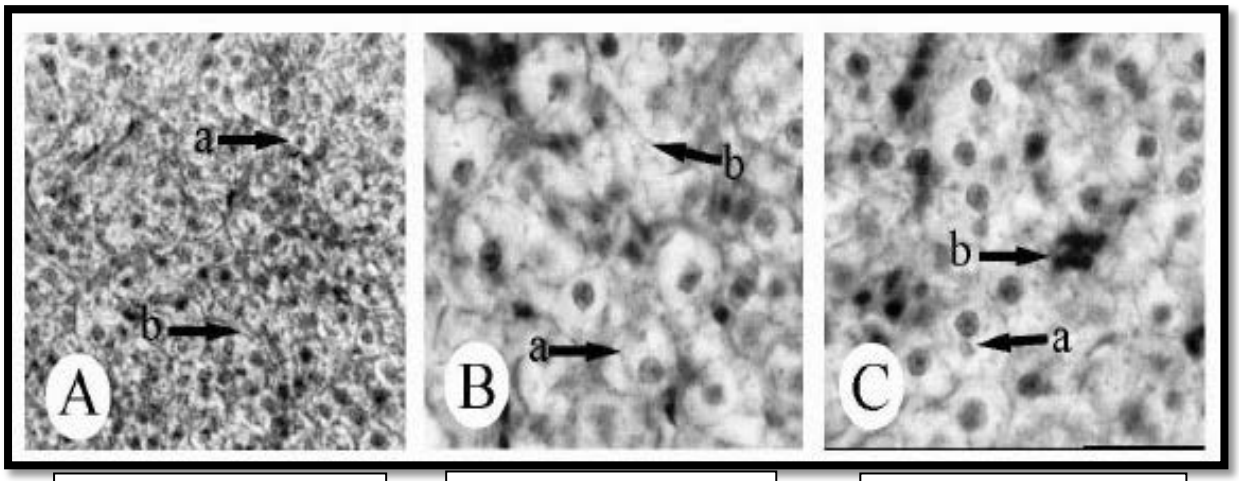
D. Nuclear degeneration (arrows) and cytoplasmic degeneration (*).Scale bar 10mm, H&E.



E. Melanomacrophages aggregate, close to a vessel (white arrow) and cytoplasmic vacuolation (*). Scale bar 10mm, H&E.

F. Hepatic tissue showing focal necrosis (white arrow). Scale bar 10mm, H&E.

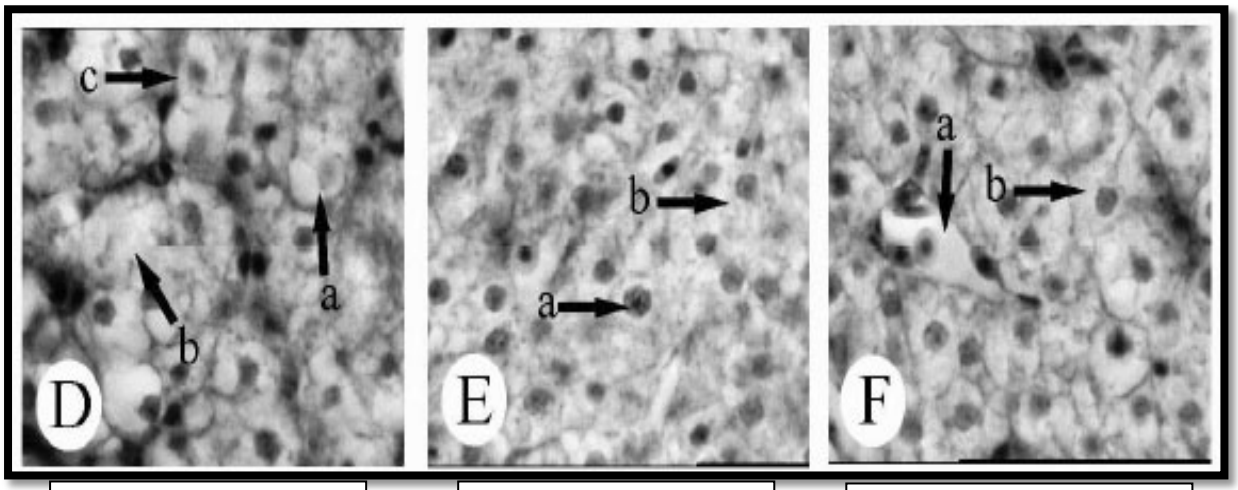
Figure 1: Photomicrographs of the liver of *P. lineatus*. Modified from Camargo and Martinez, 2007.



A. Liver tissue showing (a) hepatocyte and (b) sinusoid. H&E, X 250.

B. Liver affected by (a) Cloudy swelling of hepatocytes, (b) focal necrosis. H&E, x 400.

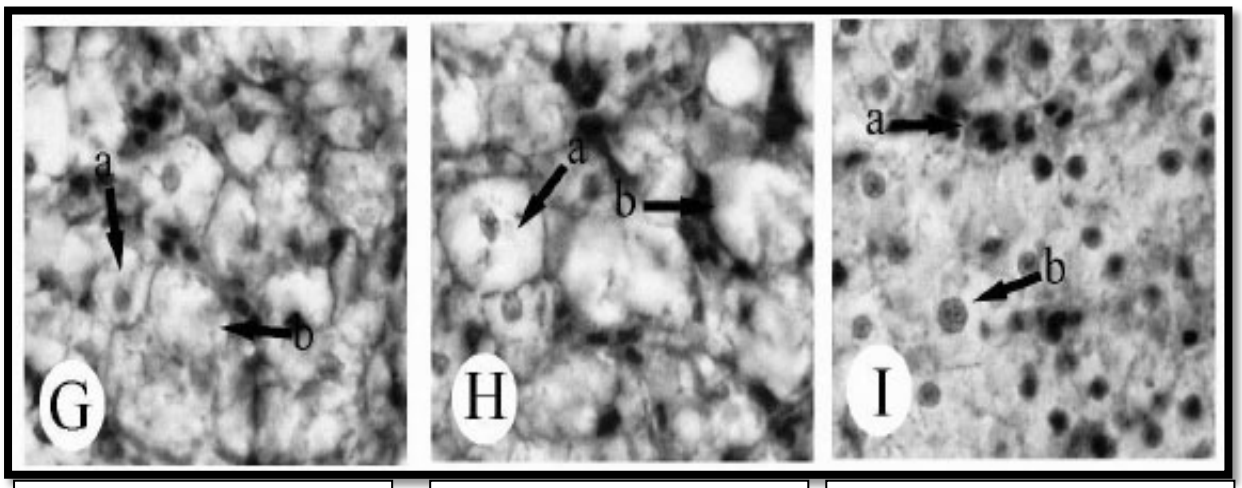
C. Liver with (a) vacuolar degeneration, (b) congestion. H&E, x 400.



D. Liver with (a) karyoliysis, (b) focal necrosis, (c) karyohexis. H&E, x 400.

E. Liver with (a) karyohexis, (b) karyolysis. H&E, x 400.

F. Liver showing (a) dilatation of sinusoids, (b) cloudy swelling of hepatocytes. H&E, x 400.



G. Liver with (a) karyoliysis, (b) focal necrosis, (c) karyohexis. H&E, x 400.

H. Liver with (a) swelling of hepatocyte, (b) congestion. H&E, x 400.

I. Liver with (a) nuclear hypertrophy, (b) congestion. H&E, x 400.

Figure 2: Liver tissue of *C.mrigala* modified from Velmurugan, Selvanayagam et al., 2009.

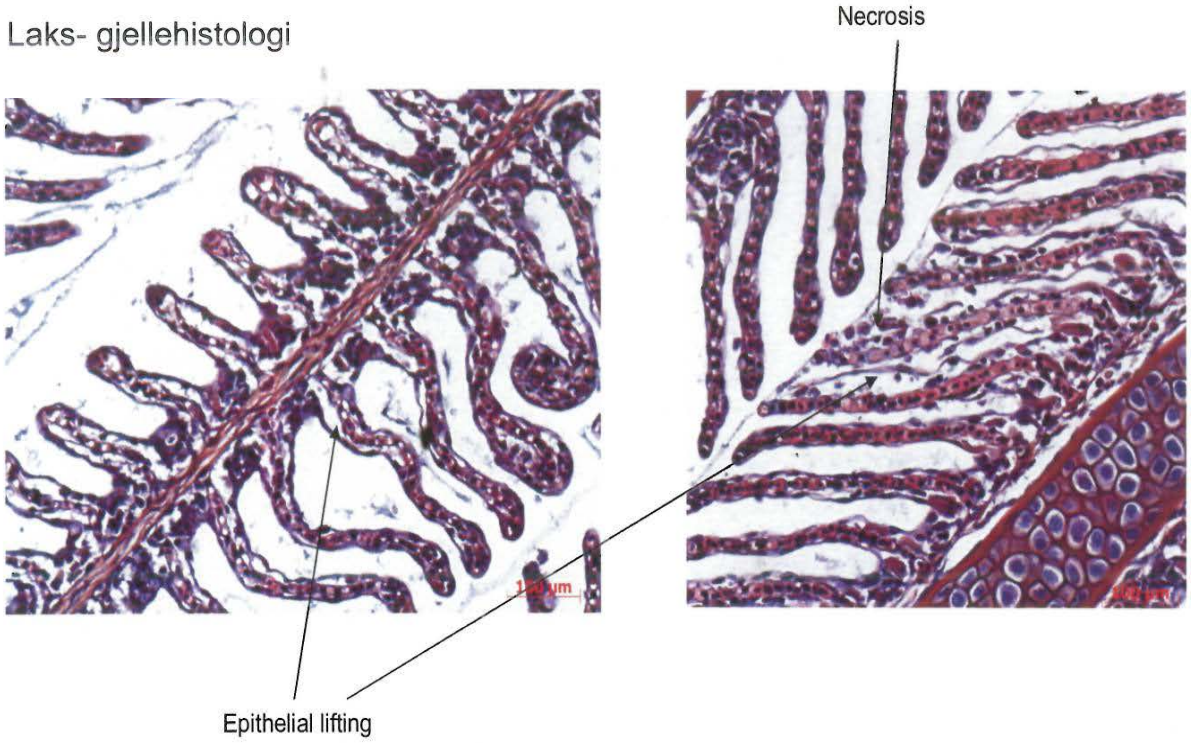
REFERENCES

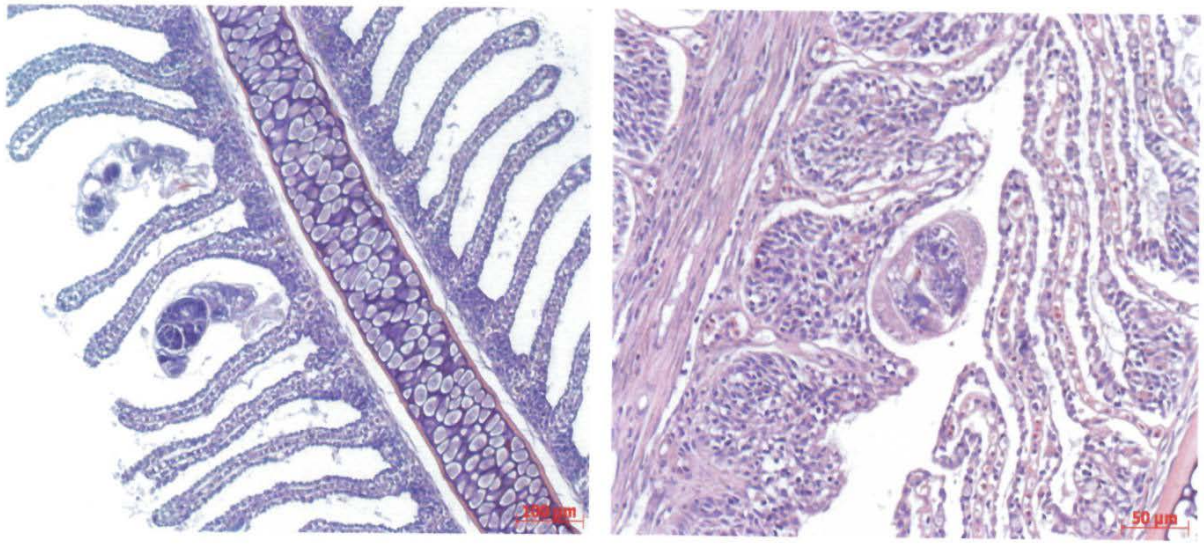
1. CAMARGO, M.M. and C. B. Martinez (2007). "Histopathology of gills, kidney and liver of a Neotropical fish caged in an urban stream." Neotropical Ichthyology 5(3) : 327-336.

2. VELMURUGAN, B., et al. 2009. "Histopathological changes in the gill and liver tissues of freshwater fish, *Cirrhinus mrigala* exposed to dichlorvos." "Brazilian Archives of Biology and Technology 52(5): 1291-1296.

GILLS
HISTOPATHOLOGY
ATLAS

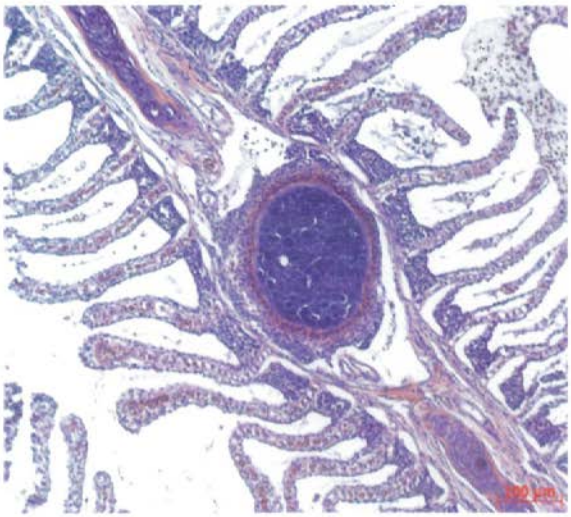
Source: Gill histopathology atlas was provided by IRIS-Biomiljø (Mekjarvik).



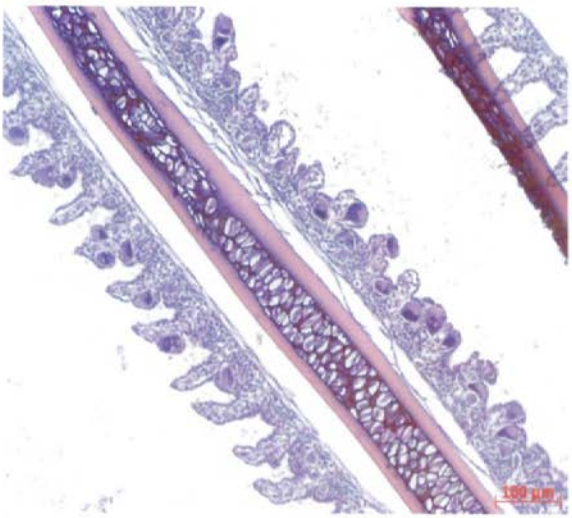


Gyrodactylus (trolig G. marinus)

Parasitter i torskegjeller

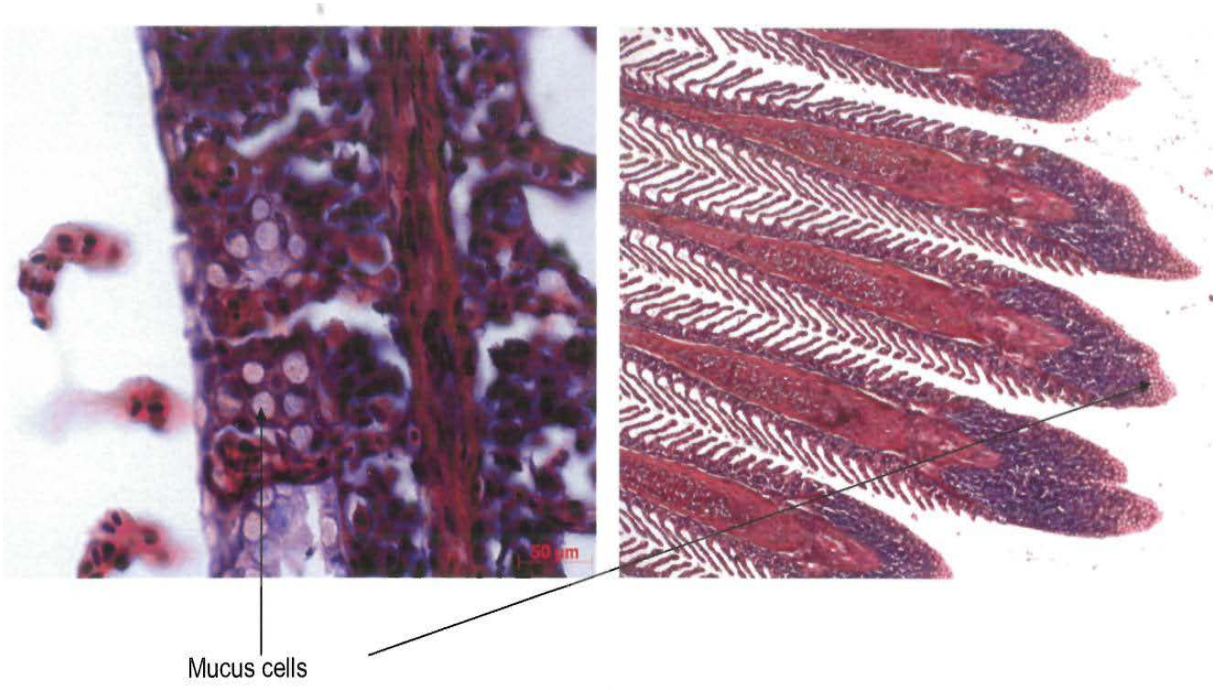


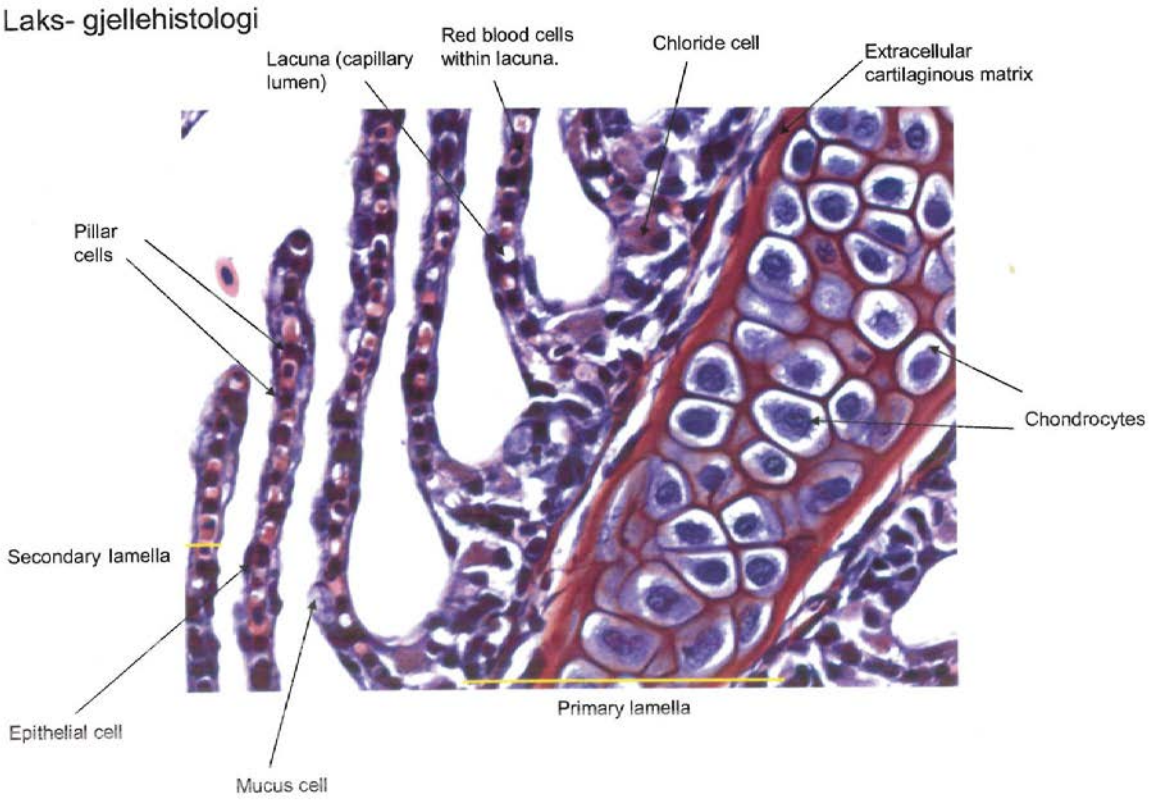
Myxosporidiae cyst

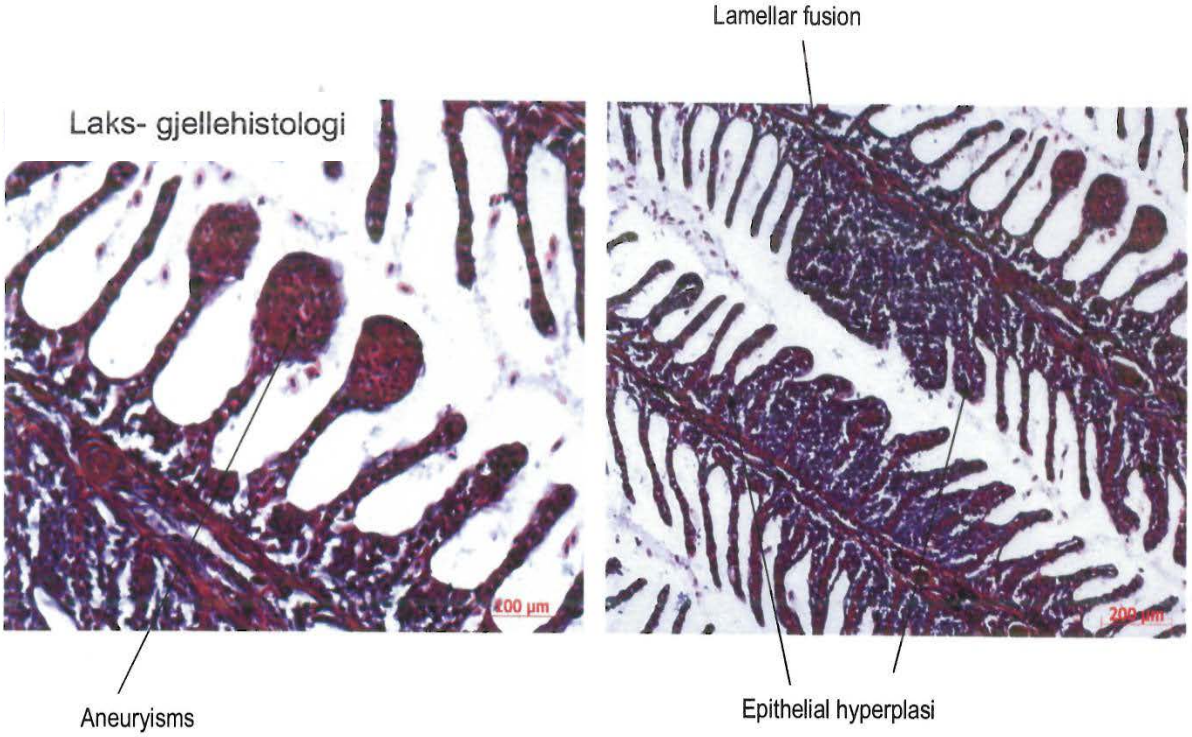


Epiteliocystis

Laks- gjellehistologi




Laks- gjellehistologi
Lacuna (capillary lumen)
Red blood cells within lacuna.
Chloride cell
Extracellular cartilaginous matrix
Pillar cells
Chondrocytes
Secondary lamella
Epithelial cell
Mucus cell
Primary lamella


Lamellar fusion
Laks- gjellehistologi
Aneuryisms
Epithelial hyperplasi

CF = 100 x Body weight (g) / (length (cm))^3

	03 DAYS			
	weight	Lenght	Lenght (cubic)	CF
C	96.00	22.00	10648.00	0.90
	103.30	22.50	11390.63	0.91
	85.90	21.50	9938.38	0.86
	126.30	25.00	15625.00	0.81
	129.90	23.00	12167.00	1.07
	96.30	21.00	9261.00	1.04
	113.40	22.00	10648.00	1.06
	86.10	22.00	10648.00	0.81
	110.70	23.00	12167.00	0.91
	90.00	22.00	10648.00	0.85
High Treated	86.50	21.00	9261.00	0.93
	102.50	22.00	10648.00	0.96
	111.70	23.00	12167.00	0.92
	102.70	22.00	10648.00	0.96
	134.60	23.00	12167.00	1.11
	121.80	23.00	12167.00	1.00
	120.00	23.00	12167.00	0.99
	95.00	22.00	10648.00	0.89
	99.20	22.00	10648.00	0.93
	123.70	24.00	13824.00	0.89
High Untreated	118.10	24.00	13824.00	0.85
	90.80	22.00	10648.00	0.85
	95.90	21.00	9261.00	1.04
	87.30	21.00	9261.00	0.94
	97.40	20.00	8000.00	1.22
	99.00	21.00	9261.00	1.07
	92.60	21.00	9261.00	1.00
	92.00	20.00	8000.00	1.15
	81.10	20.00	8000.00	1.01
	97.40	21.00	9261.00	1.05
Low Untreated	94.20	22.00	10648.00	0.88
	121.30	23.00	12167.00	1.00
	106.60	22.00	10648.00	1.00
	127.40	24.00	13824.00	0.92
	104.70	22.00	10648.00	0.98
	88.00	21.00	9261.00	0.95
	86.20	19.00	6859.00	1.26
	92.40	21.00	9261.00	1.00
	112.20	23.00	12167.00	0.92
	93.10	22.00	10648.00	0.87
Low Treated	81.50	21.00	9261.00	0.88
	86.00	21.00	9261.00	0.93
	97.30	21.50	9938.38	0.98
	102.20	22.00	10648.00	0.96
	117.70	23.00	12167.00	0.97
	104.50	22.00	10648.00	0.98
	113.70	23.00	12167.00	0.93
	114.60	23.00	12167.00	0.94
	85.70	22.00	10648.00	0.80
	82.70	21.00	9261.00	0.89

	07 DAYS			
	weight	lenght	Lenght (cubic)	CF
C	92.20	22.00	10648.00	0.87
	131.70	24.00	13824.00	0.95
	112.20	23.00	12167.00	0.92
	91.60	21.00	9261.00	0.99
	103.60	22.50	10648.00	0.97
	83.60	21.00	9261.00	0.90
	97.00	21.00	9261.00	1.05
	88.60	21.50	9938.38	0.89
	97.00	22.00	10648.00	0.91
	111.30	23.00	12167.00	0.91
HT	68.60	20.00	8000.00	0.86
	127.90	24.50	14706.13	0.87
	99.60	21.00	9261.00	1.08
	103.50	22.50	11390.63	0.91
	83.40	21.00	9261.00	0.90
	73.50	20.50	8615.13	0.85
	102.60	22.50	11390.63	0.90
	83.80	21.00	9261.00	0.90
	106.40	23.00	12167.00	0.87
	123.70	23.00	12167.00	1.02
HU	94.40	22.00	10648.00	0.89
	87.60	22.00	10648.00	0.82
	83.20	21.00	9261.00	0.90
	92.60	22.00	10648.00	0.87
	104.20	22.50	11390.63	0.91
	123.40	24.00	13824.00	0.89
	116.00	23.00	12167.00	0.95
	112.00	22.50	11390.63	0.98
	94.20	21.50	9938.38	0.95
	114.30	23.50	12977.88	0.88
LU	128.00	24.00	13824.00	0.93
	90.20	23.00	12167.00	0.74
	88.40	21.50	9938.38	0.89
	133.40	25.00	15625.00	0.85
	79.20	21.00	9261.00	0.86
	96.40	22.00	10648.00	0.91
	85.40	21.00	9261.00	0.92
	94.60	21.50	9938.38	0.95
	87.90	21.00	9261.00	0.95
	82.90	21.50	9938.38	0.83
LT	83.90	21.00	9261.00	0.91
	140.10	24.50	14706.13	0.95
	84.90	21.00	9261.00	0.92
	114.10	24.00	13824.00	0.83
	98.00	22.00	10648.00	0.92
	100.00	22.00	10648.00	0.94
	81.70	21.00	9261.00	0.88
	124.50	24.00	13824.00	0.90
	77.80	21.00	9261.00	0.84
	101.40	22.00	10648.00	0.95

	14 DAYS			
	lenght	weight	Lenght (cubic)	CF
C	25.00	113.20	15625.00	0.72
	20.00	81.20	8000.00	1.02
	21.00	97.30	9261.00	1.05
	22.00	105.40	10648.00	0.99
	22.00	97.50	10648.00	0.92
	24.00	141.30	13824.00	1.02
	25.00	147.20	15625.00	0.94
	22.00	94.40	10648.00	0.89
	24.00	128.80	13824.00	0.93
	21.00	95.50	9261.00	1.03
HT	24.50	119.50	13824.00	0.86
	23.00	117.60	12167.00	0.97
	21.00	86.20	9261.00	0.93
	25.00	125.80	15625.00	0.81
	23.00	103.50	12167.00	0.85
	23.50	117.70	12167.00	0.97
	23.50	109.90	12167.00	0.90
	26.00	156.60	17576.00	0.89
	23.00	101.20	12167.00	0.83
	21.00	82.30	9261.00	0.89
HU	24.00	109.40	13824.00	0.79
	25.00	145.20	15625.00	0.93
	22.50	98.00	10648.00	0.92
	21.00	95.00	9261.00	1.03
	22.00	91.50	10648.00	0.86
	22.00	102.30	10648.00	0.96
	25.00	144.40	15625.00	0.92
	22.00	79.20	10648.00	0.74
	23.00	130.00	12167.00	1.07
	23.00	109.10	12167.00	0.90
LU	23.00	111.90	12167.00	0.92
	20.00	91.20	8000.00	1.14
	22.00	102.00	10648.00	0.96
	22.00	115.00	10648.00	1.08
	22.00	102.00	10648.00	0.96
	22.00	106.00	10648.00	1.00
	21.00	92.90	9261.00	1.00
	22.00	112.50	10648.00	1.06
	22.00	108.30	10648.00	1.02
	22.00	106.80	10648.00	1.00
LT	24.00	126.60	13824.00	0.92
	21.00	95.80	9261.00	1.03
	22.00	90.10	10648.00	0.85
	20.00	74.90	8000.00	0.94
	22.00	95.90	10648.00	0.90
	23.00	115.00	12167.00	0.95
	21.00	86.80	9261.00	0.94
	22.00	94.70	10648.00	0.89
	21.00	93.00	9261.00	1.00
	22.00	101.20	10648.00	0.95

LSI = (Liver weight (g) / Fish weight (g)) × 100

03 DAYS					
Fish Number	Tank/Esposure	Fish weight (g)	Liver weight (g)	LSI	
1	control	96,00	0,5	0,52	C
2	control	103,30	0,95	0,92	
3	control	85,90	0,73	0,85	
4	control	126,30	1,22	0,97	
5	control	129,90	0,74	0,57	
6	control	96,30	0,84	0,87	
7	control	113,40	0,87	0,77	
8	control	86,10	0,56	0,65	
9	control	110,70	0,82	0,74	
10	control	90,00	0,9	1,00	
11	Tank 1	86,50	0,64	0,74	HT
12	Tank 1	102,50	1,03	1,00	
13	Tank 1	111,70	0,99	0,89	
14	Tank 1	102,70	0,68	0,66	
15	Tank 1	134,60	0,2	0,15	
16	Tank 1	121,80	0,95	0,78	
17	Tank 1	120,00	0,98	0,82	
18	Tank 1	95,00	0,84	0,88	
19	Tank 1	99,20	0,8	0,81	
20	Tank 1	123,70	1,26	1,02	
21	Tank 2	118,10	1,02	0,86	HU
22	Tank 2	90,80	0,69	0,76	
23	Tank 2	95,90	0,92	0,96	
24	Tank 2	87,30	0,72	0,82	
25	Tank 2	97,40	1,33	1,37	
26	Tank 2	99,00	0,72	0,73	
27	Tank 2	92,60	0,89	0,96	
28	Tank 2	92,00	0,93	1,01	
29	Tank 2	81,10	0,71	0,88	
30	Tank 2	97,40	0,79	0,81	
31	Tank 3	94,20	0,96	1,02	LU
32	Tank 3	121,30	1,13	0,93	
33	Tank 3	106,60	1,05	0,98	
34	Tank 3	127,40	1,03	0,81	
35	Tank 3	104,70	0,92	0,88	
36	Tank 3	88,00	0,69	0,78	
37	Tank 3	86,20	0,79	0,92	
38	Tank 3	92,40	0,68	0,74	
39	Tank 3	112,20	1,16	1,03	
40	Tank 3	93,10	0,77	0,83	
41	Tank4	81,50	0,76	0,93	LT
42	Tank4	86,00	0,63	0,73	
43	Tank4	97,30	0,69	0,71	
44	Tank4	102,20	0,63	0,62	
45	Tank4	117,70	0,66	0,56	
46	Tank4	104,50	0,99	0,95	
47	Tank4	113,70	0,95	0,84	
48	Tank4	114,60	0,8	0,70	
49	Tank4	85,70	0,77	0,90	
50	Tank4	82,70	0,76	0,92	

LSI = (Liver weight (g) / Fish weight (g)) × 100

07 DAYS					
Fish Number	**Tank/Esposure**	**Fish weight (g)**	**Liver weight (g)**	**LSI**	
51	control	92,20	0,64	0,69	**C**
52	control	131,70	1,34	1,02	
53	control	112,20	0,91	0,81	
54	control	91,60	0,76	0,83	
55	control	103,60	0,82	0,79	
56	control	83,60	0,72	0,86	
57	control	97,00	0,68	0,70	
58	control	88,60	0,66	0,74	
59	control	97,00	0,7	0,72	
60	control	111,30	0,89	0,80	
61	Tank 1	68,60	0,69	1,01	**HT**
62	Tank 1	127,90	0,91	0,71	
63	Tank 1	99,60	0,77	0,77	
64	Tank 1	103,50	0,83	0,80	
65	Tank 1	83,40	0,76	0,91	
66	Tank 1	73,50	0,69	0,94	
67	Tank 1	102,60	0,73	0,71	
68	Tank 1	83,80	0,63	0,75	
69	Tank 1	106,40	1,23	1,16	
70	Tank 1	123,70	1,09	0,88	
71	Tank 2	94,40	0,79	0,84	**HU**
72	Tank 2	87,60	0,59	0,67	
73	Tank 2	83,20	0,78	0,94	
74	Tank 2	92,60	0,79	0,85	
75	Tank 2	104,20	0,64	0,61	
76	Tank 2	123,40	1,07	0,87	
77	Tank 2	116,00	0,86	0,74	
78	Tank 2	112,00	1,16	1,04	
79	Tank 2	94,20	0,49	0,52	
80	Tank 2	114,30	0,59	0,52	
81	Tank 3	128,00	1,01	0,79	**LU**
82	Tank 3	90,20	0,75	0,83	
83	Tank 3	88,40	0,79	0,89	
84	Tank 3	133,40	1,32	0,99	
85	Tank 3	79,20	0,7	0,88	
86	Tank 3	96,40	1,15	1,19	
87	Tank 3	85,40	0,61	0,71	
88	Tank 3	94,60	0,72	0,76	
89	Tank 3	87,90	0,76	0,86	
90	Tank 3	82,90	0,71	0,86	
91	Tank4	83,90	0,61	0,73	**LT**
92	Tank4	140,10	0,96	0,69	
93	Tank4	84,90	0,75	0,88	
94	Tank4	114,10	0,96	0,84	
95	Tank4	98,00	0,8	0,82	
96	Tank4	100,00	0,81	0,81	
97	Tank4	81,70	0,67	0,82	
98	Tank4	124,50	0,87	0,70	
99	Tank4	77,80	0,55	0,71	
100	Tank4	101,40	0,7	0,69	

LSI = (Liver weight (g) / Fish weight (g)) × 100

14 DAYS					
Fish Number	**Tank/Esposure**	**Fish weight (g)**	**Liver weight (g)**	**LSI**	
101	control	113,20	-		
102	control	81,20	0,61	0,75	
103	control	97,30	0,65	0,67	
104	control	105,40	1,26	1,20	
105	control	97,50	0,67	0,69	**C**
106	control	141,30	1,28	0,91	
107	control	147,20	1,29	0,88	
108	control	94,40	0,8	0,85	
109	control	128,80	1	0,78	
110	control	95,50	0,8	0,84	
111	Tank 1	119,50	0,87	0,73	
112	Tank 1	117,60	1,15	0,98	
113	Tank 1	86,20	0,57	0,66	
114	Tank 1	125,80	1,21	0,96	
115	Tank 1	103,50	0,82	0,79	**HT**
116	Tank 1	117,70	1	0,85	
117	Tank 1	109,90	1	0,91	
118	Tank 1	156,60	1,43	0,91	
119	Tank 1	101,20	0,84	0,83	
120	Tank 1	82,30	0,68	0,83	
121	Tank 2	109,40	0,89	0,81	
122	Tank 2	145,20	1,23	0,85	
123	Tank 2	98,00	1,11	1,13	
124	Tank 2	95,00	0,7	0,74	
125	Tank 2	91,50	0,67	0,73	**HU**
126	Tank 2	102,30	0,84	0,82	
127	Tank 2	144,40	1,02	0,71	
128	Tank 2	79,20	0,38	0,48	
129	Tank 2	130,00	1,17	0,90	
130	Tank 2	109,10	0,84	0,77	
131	Tank 3	111,90	1,02	0,91	
132	Tank 3	91,20	0,89	0,98	
133	Tank 3	102,00	0,65	0,64	
134	Tank 3	115,00	0,93	0,81	
135	Tank 3	102,00	0,68	0,67	**LU**
136	Tank 3	106,00	1	0,94	
137	Tank 3	92,90	0,74	0,80	
138	Tank 3	112,50	0,85	0,76	
139	Tank 3	108,30	0,92	0,85	
140	Tank 3	106,80	0,81	0,76	
141	Tank4	126,60	0,99	0,78	
142	Tank4	95,80	0,65	0,68	
143	Tank4	90,10	0,62	0,69	
144	Tank4	74,90	0,57	0,76	
145	Tank4	95,90	0,86	0,90	**LT**
146	Tank4	115,00	0,82	0,71	
147	Tank4	86,80	0,72	0,83	
148	Tank4	94,70	0,78	0,82	
149	Tank4	93,00	0,68	0,73	
150	Tank4	101,20	0,91	0,90	

GILLS HISTOPATHOLOGICAL ALTERATIONS

0: Normal | 1: Mild | 2: Mild to moderate | 3: Moderate | 4: Severe

ID	Treatment	AN	EP	EH	LF	LC	HPC	CCD	BG	EMS	Nec	PEC	
101	control	**0**	**2**	**0**	**0**	**2**	**0**	**0**	**2**	**0**	**0**	**2**	REFERENCE
102	control	**1**	**1**	**2**	**0**	**0**	**0**	**0**	**1**	**1**	**0**	**1**	
103	control	**0**	**0**	**0**	**2**	**0**	**0**	**0**	**2**	**0**	**0**	**3**	
104	control	**2**	**1**	**3**	**2**	**0**	**0**	**0**	**2**	**1**	**2**	**1**	
105	control	**0**	**0**	**3**	**0**	**0**	**0**	**0**	**3**	**1**	**0**	**2**	
106	control	**1**	**1**	**2**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**1**	
107	control	**0**	**0**	**3**	**1**	**0**	**0**	**0**	**3**	**2**	**0**	**2**	
	Mean	**0,57**	**0,71**	**1,86**	**0,71**	**0,29**	**0,00**	**0,00**	**2,00**	**0,71**	**0,29**	**1,71**	
	SD	**0,79**	**0,76**	**1,35**	**0,95**	**0,76**	**0,00**	**0,00**	**0,82**	**0,76**	**0,76**	**0,76**	
	CV (%)	**137,69**	**105,83**	**72,43**	**133,17**	**264,58**	**0,00**	**0,00**	**40,82**	**105,83**	**264,58**	**44,10**	

ID	Treatment	AN	EP	EH	LF	LC	HPC	CCD	BG	EMS	Nec	PEC	
111	Tank 1	**0**	**1**	**2**	**0**	**0**	**0**	**0**	**3**	**2**	**0**	**2**	HIGH TREATED
112	Tank 1	**1**	**3**	**1**	**0**	**2**	**0**	**0**	**4**	**3**	**0**	**2**	
113	Tank 1	**3**	**2**	**3**	**2**	**1**	**0**	**0**	**3**	**2**	**2**	**3**	
114	Tank 1	**4**	**3**	**3**	**3**	**2**	**0**	**0**	**3**	**4**	**3**	**4**	
115	Tank 1	**4**	**3**	**2**	**2**	**2**	**0**	**0**	**4**	**3**	**0**	**3**	
116	Tank 1	**4**	**3**	**2**	**2**	**0**	**0**	**0**	**4**	**3**	**3**	**3**	
117	Tank 1	**0**	**1**	**2**	**0**	**0**	**0**	**0**	**1**	**2**	**0**	**2**	
	Median	**3,00**	**3,00**	**2,00**	**2,00**	**1,00**	**0,00**	**0,00**	**3,00**	**3,00**	**0,00**	**3,00**	
	Mean	**2,29**	**2,29**	**2,14**	**1,29**	**1,00**	**0,00**	**0,00**	**3,14**	**2,71**	**1,14**	**2,71**	
	SD	**1,89**	**0,95**	**0,69**	**1,25**	**1,00**	**0,00**	**0,00**	**1,07**	**0,76**	**1,46**	**0,76**	
	CV (%)	**82,68**	**41,61**	**32,20**	**97,50**	**100,00**	**0,00**	**0,00**	**34,02**	**27,85**	**128,09**	**27,85**	

ID	Treatment	AN	EP	EH	LF	LC	HPC	CCD	BG	EMS	Nec	PEC	
121	Tank 2	**4**	**3**	**2**	**2**	**0**	**0**	**0**	**4**	**3**	**3**	**2**	HIGH UNTREATED
122	Tank 2	**4**	**4**	**2**	**3**	**1**	**0**	**0**	**4**	**3**	**4**	**2**	
123	Tank 2	**4**	**4**	**3**	**2**	**0**	**0**	**0**	**4**	**4**	**3**	**3**	
124	Tank 2	**4**	**3**	**3**	**0**	**0**	**0**	**0**	**4**	**3**	**4**	**3**	
125	Tank 2	**2**	**4**	**3**	**3**	**0**	**0**	**0**	**4**	**3**	**2**	**1**	
126	Tank 2	**4**	**3**	**1**	**2**	**0**	**0**	**0**	**4**	**3**	**3**	**4**	
127	Tank 2	**0**	**0**	**2**	**0**	**2**	**0**	**0**	**4**	**2**	**0**	**2**	
	Median	**4,00**	**3,00**	**2,00**	**2,00**	**0,00**	**0,00**	**0,00**	**4,00**	**3,00**	**3,00**	**2,00**	
	Mean	**3,14**	**3,00**	**2,29**	**1,71**	**0,43**	**0,00**	**0,00**	**4,00**	**3,00**	**2,71**	**2,43**	
	SD	**1,57**	**1,41**	**0,76**	**1,25**	**0,79**	**0,00**	**0,00**	**0,00**	**0,58**	**1,38**	**0,98**	
	CV (%)	**50,07**	**47,14**	**33,07**	**73,12**	**183,59**	**0,00**	**0,00**	**0,00**	**19,25**	**50,85**	**40,18**	

ID	Treatment	AN	EP	EH	LF	LC	HPC	CCD	BG	EMS	Nec	PEC	
131	Tank 3	**0**	**0**	**2**	**1**	**1**	**0**	**0**	**2**	**1**	**0**	**2**	LOW UNTREATED
132	Tank 3	**0**	**0**	**1**	**0**	**2**	**0**	**0**	**2**	**0**	**0**	**0**	
133	Tank 3	**0**	**0**	**2**	**2**	**0**	**0**	**0**	**0**	**3**	**1**	**2**	
134	Tank 3	**3**	**2**	**0**	**2**	**3**	**0**	**0**	**2**	**0**	**0**	**0**	
135	Tank 3	**1**	**0**	**2**	**2**	**0**	**0**	**0**	**0**	**3**	**0**	**1**	
136	Tank 3	**0**	**1**	**2**	**2**	**0**	**0**	**0**	**1**	**2**	**0**	**3**	
137	Tank 3	**4**	**2**	**0**	**3**	**0**	**0**	**0**	**3**	**2**	**0**	**0**	
	Median	**0,00**	**0,00**	**2,00**	**2,00**	**0,00**	**0,00**	**0,00**	**2,00**	**2,00**	**0,00**	**1,00**	
	Mean	**1,14**	**0,71**	**1,29**	**1,71**	**0,86**	**0,00**	**0,00**	**1,43**	**1,57**	**0,14**	**1,14**	
	SD	**1,68**	**0,95**	**0,95**	**0,95**	**1,21**	**0,00**	**0,00**	**1,13**	**1,27**	**0,38**	**1,21**	
	CV (%)	**146,66**	**133,17**	**73,98**	**55,49**	**141,75**	**0,00**	**0,00**	**79,37**	**80,97**	**264,58**	**106,31**	

XX

ID	Treatment	AN	EP	EH	LF	LC	HPC	CCD	BG	EMS	Nec	PEC	
141	Tank4	**0**	**2**	**1**	**0**	**0**	**0**	**0**	**1**	**1**	**0**	**1**	LOW TREATED
142	Tank4	**0**	**0**	**1**	**3**	**0**	**0**	**0**	**0**	**1**	**0**	**3**	
143	Tank4	**1**	**0**	**1**	**0**	**0**	**0**	**0**	**1**	**0**	**3**	**2**	
144	Tank4	**3**	**2**	**2**	**1**	**0**	**0**	**0**	**3**	**2**	**2**	**3**	
145	Tank4	**0**	**0**	**2**	**1**	**0**	**0**	**0**	**0**	**1**	**0**	**2**	
146	Tank4	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	
147	Tank4	**0**	**0**	**1**	**2**	**0**	**0**	**0**	**0**	**1**	**0**	**2**	
	Median	**0,00**	**0,00**	**1,00**	**1,00**	**0,00**	**0,00**	**0,00**	**0,00**	**1,00**	**0,00**	**2,00**	
	Mean	**0,86**	**0,57**	**1,14**	**1,00**	**0,00**	**0,00**	**0,00**	**0,71**	**0,86**	**0,71**	**2,14**	
	SD	**1,21**	**0,98**	**0,69**	**1,15**	**0,00**	**0,00**	**0,00**	**1,11**	**0,69**	**1,25**	**0,69**	
	CV (%)	**141,75**	**170,78**	**60,38**	**115,47**	**0,00**	**0,00**	**0,00**	**155,78**	**80,51**	**175,50**	**32,20**	

	MEAN SUMMARY										
	AN	EP	EH	LF	LC	HPC	CCD	BG	EMS	Nec	PEC
Control	0,57	0,71	1,86	0,71	0,29	2,00	0,71	0,29	1,71	0,00	0,00
High treated	2,29	2,29	2,14	1,29	1,00	3,14	2,71	1,14	2,71	0,00	0,00
High Untreated	3,14	3,00	2,29	1,71	0,43	4,00	3,00	2,71	2,43	0,00	0,00
Low Untreated	1,14	0,71	1,29	1,71	0,86	1,43	1,57	0,14	1,14	0,00	0,00
Low treated	0,86	0,57	1,14	1,00	0,00	0,71	0,86	0,71	2,14	0,00	0,00

	SD SUMMARY										
	AN	EP	EH	LF	LC	HPC	CCD	BG	EMS	Nec	PEC
Control	0,79	0,76	1,35	0,95	0,76	0,82	0,76	0,76	0,76	0,00	0,00
High treated	1,89	0,95	0,69	1,25	1,00	1,07	0,76	1,46	0,76	0,00	0,00
High Untreated	1,57	1,41	0,76	1,25	0,79	0,00	0,58	1,38	0,98	0,00	0,00
Low Untreated	1,68	0,95	0,95	0,95	1,21	1,13	1,27	0,38	1,21	0,00	0,00
Low treated	1,21	0,98	0,69	1,15	0,00	1,11	0,69	1,25	0,69	0,00	0,00

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att: Daniela Pampanin

Our ref: 2014-02204
Edition: 1
Date: 15.apr.2014
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Your ref: Daniela M. Pampanin

Laboratory Report

Objective:	PAH, OIW, heavy metals incl. mercury in water samples.
Sampling location:	n/a
Sampled by:	International Research Institute of Stavanger
Received date:	18.mar.2014
Tested:	March-April 2014

If you should have any questions to the report, please do not hesitate to contact us.

Regards
Intertek West Lab AS

Technical responsible

Torbjørn Tyvold
Dept Man Environment and Processes
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Analysed by

Terese Lima Bertram
Team Leader
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Intertek

Laboratory Report

Sample marking	Tank 1 - PAH	
Sampled Date	17.mar.2014	15:30:00
Sample type	Drainwater	

Results for sample 2014-02204-001

Parameter	Results	Unit	PQL Lower	PQL Upper	Method/standard	Uncertainty Rel | Abs
PAH/NPD in water, GC/MS						
Naphtalene	0,03	µg/l	0,01		ISO28540:2011	30% | ±0,02
Sum C1-Naphtalene	0,06	µg/l	0,01		ISO28540:2011	35% | ±0,02
Sum C2-Naphtalene	<0,04	µg/l	0,01		ISO28540:2011	35% | ±0,02
Sum C3 Naphtalene	<0,04	µg/l	0,01		ISO28540:2011	40% | ±0,08
Acenaphtylene	<0,01	µg/l	0,01		ISO28540:2011	30% | ±0,02
Acenaphtene	<0,01	µg/l	0,01		ISO28540:2011	30% | ±0,02
Fluorene	<0,01	µg/l	0,01		ISO28540:2011	30% | ±0,02
Phenanthrene	<0,01	µg/l	0,01		ISO28540:2011	30% | ±0,02
Anthracene	<0,02	µg/l	0,02		ISO28540:2011	50% | ±0,05
Sum C1-Phenantrene/Antracene	n,a	µg/l	0,01		ISO28540:2011	35% | ±0,02
Sum C2-Phenantrene/Antracene	n,a	µg/l	0,01		ISO28540:2011	40% | ±0,08
Sum C3-Phenantrene/Antracene	n,a	µg/l	0,01		ISO28540:2011	50% | ±0,15
Dibenzothiophene	<0,01	µg/l	0,01		ISO28540:2011	30% | ±0,02
Sum C1-dibenzothiophenes	n,a	µg/l	0,01		ISO28540:2011	30% | ±0,02
Sum C2-Dibenzotiophene	n,a	µg/l	0,01		ISO28540:2011	40% | ±0,03
Sum C3-Dibenzotiophene	n,a	µg/l	0,01		ISO28540:2011	40% | ±0,08
Fluoranthene	<0,02	µg/l	0,02		ISO28540:2011	35% | ±0,05
Pyrene	<0,01	µg/l	0,01		ISO28540:2011	30% | ±0,02
Benzo(a)anthracene	<0,01	µg/l	0,01		ISO28540:2011	35% | ±0,02
Chrycene	<0,01	µg/l	0,01		ISO28540:2011	30% | ±0,02
Benzo(b)fluoranthene	<0,02	µg/l	0,02		ISO28540:2011	35% | ±0,05
Benzo(k)fluoranthene	<0,01	µg/l	0,01		ISO28540:2011	30% | ±0,02
Indeno(1,2,3-cd)pyrene	<0,02	µg/l	0,02		ISO28540:2011	40% | ±0,04
Benzo(ghi)perylene	<0,01	µg/l	0,01		ISO28540:2011	35% | ±0,02
Benzo(a)pyrene	<0,01	µg/l	0,01		ISO28540:2011	30% | ±0,03
Dibenzo(ah)anthracene	<0,01	µg/l	0,01		ISO28540:2011	35% | ±0,02
Sum 16 EPA-PAH	0,03	µg/l			ISO28540:2011	n.a | n.a
Sum NPD	0,09	µg/l			ISO28540:2011	n.a | n.a

Explanation: PQL = Practical Quantification limit. # = The analysis is performed by sub contractor.

The uncertainty is expressed at 95% confidence level. If both a relative and an absolute uncertainty argument is stated, it is the argument that represents the highest uncertainty that applies.

Intertek

Laboratory Report

Sample marking	Tank 1 - Metaller	
Sampled Date	17.mar.2014	15:30:00
Sample type	Drainwater	

Results for sample 2014-02204-002

Parameter	Results	Unit	PQL Lower Upper	Method/standard	Uncertainty Rel | Abs
Metals in seawater, ICP-MS					
Arsenic, As	<1,0	µg/l	1,0 5000	Basert på EPA200.8	15% | ±3,0
Barium, Ba	440	µg/l	10 1000000	Basert på EPA200.8	20% | ±30
Cadmium, Cd	<0,15	µg/l	0,15 5000	Basert på EPA200.8	15% | ±0,45
Nickel,Ni	<1,5	µg/l	1,5 5000	Basert på EPA200.8	20% | ±4,5
Chromium, Cr	<0,4	µg/l	0,4 5000	Basert på EPA200.8	20% | ±1,2
Copper, Cu	0,63	µg/l	0,5 5000	Basert på EPA200.8	30% | ±1,5
Iron, Fe	58	µg/l	20 400000	Basert på EPA200.8	15% | ±60
Lead, Pb	0,31	µg/l	0,25 5000	Basert på EPA200.8	20% | ±0,75
Zinc, Zn	<4	µg/l	4 1000000	Basert på EPA200.8	25% | ±12

Explanation: PQL = Practical Quantification limit. # = The analysis is performed by sub contractor.

The uncertainty is expressed at 95% confidence level. If both a relative and an absolute uncertainty argument is stated, it is the argument that represents the highest uncertainty that applies.

Intertek

Laboratory Report

Sample marking	Tank 1 - Hg	
Sampled Date	17.mar.2014	15:30:00
Sample type	Drainwater	

Results for sample 2014-02204-003

Parameter	Results	Unit	PQL Lower Upper	Method/standard	Uncertainty Rel | Abs
Mercury in water, FIMS					
Mercury, Hg	0,062	µg/l	0,01	Mod. NS-EN 1483	15% | ±0,01

Explanation: PQL = Practical Quantification limit. # = The analysis is performed by sub contractor.

The uncertainty is expressed at 95% confidence level. If both a relative and an absolute uncertainty argument is stated, it is the argument that represents the highest uncertainty that applies.

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Intertek

Laboratory Report

Sample marking	Tank 2 - PAH	
Sampled Date	17.mar.2014	15:30:00
Sample type	Drainwater	

Results for sample 2014-02204-004

Parameter	Results	Unit	PQL Lower	PQL Upper	Method/standard	Uncertainty Rel | Abs
PAH/NPD in water, GC/MS						
Naphtalene	0,12	µg/l	0,01		ISO28540:2011	30% | ±0,02
Sum C1-Naphtalene	0,30	µg/l	0,01		ISO28540:2011	35% | ±0,02
Sum C2-Naphtalene	0,26	µg/l	0,01		ISO28540:2011	35% | ±0,02
Sum C3 Naphtalene	0,25	µg/l	0,01		ISO28540:2011	40% | ±0,08
Acenaphtylene	<0,01	µg/l	0,01		ISO28540:2011	30% | ±0,02
Acenaphtene	<0,01	µg/l	0,01		ISO28540:2011	30% | ±0,02
Fluorene	<0,01	µg/l	0,01		ISO28540:2011	30% | ±0,02
Phenanthrene	<0,02	µg/l	0,01		ISO28540:2011	30% | ±0,02
Anthracene	<0,02	µg/l	0,02		ISO28540:2011	50% | ±0,05
Sum C1-Phenantrene/Antracene	n,a	µg/l	0,01		ISO28540:2011	35% | ±0,02
Sum C2-Phenantrene/Antracene	0,05	µg/l	0,01		ISO28540:2011	40% | ±0,08
Sum C3-Phenantrene/Antracene	n,a	µg/l	0,01		ISO28540:2011	50% | ±0,15
Dibenzothiophene	<0,01	µg/l	0,01		ISO28540:2011	30% | ±0,02
Sum C1-dibenzothiophenes	n,a	µg/l	0,01		ISO28540:2011	30% | ±0,02
Sum C2-Dibenzotiophene	n,a	µg/l	0,01		ISO28540:2011	40% | ±0,03
Sum C3-Dibenzotiophene	n,a	µg/l	0,01		ISO28540:2011	40% | ±0,08
Fluoranthene	<0,02	µg/l	0,02		ISO28540:2011	35% | ±0,05
Pyrene	<0,01	µg/l	0,01		ISO28540:2011	30% | ±0,02
Benzo(a)anthracene	<0,01	µg/l	0,01		ISO28540:2011	35% | ±0,02
Chrycene	<0,01	µg/l	0,01		ISO28540:2011	30% | ±0,02
Benzo(b)fluoranthene	<0,02	µg/l	0,02		ISO28540:2011	35% | ±0,05
Benzo(k)fluoranthene	<0,01	µg/l	0,01		ISO28540:2011	30% | ±0,02
Indeno(1,2,3-cd)pyrene	<0,02	µg/l	0,02		ISO28540:2011	40% | ±0,04
Benzo(ghi)perylene	<0,01	µg/l	0,01		ISO28540:2011	35% | ±0,02
Benzo(a)pyrene	<0,01	µg/l	0,01		ISO28540:2011	30% | ±0,03
Dibenzo(ah)anthracene	<0,01	µg/l	0,01		ISO28540:2011	35% | ±0,02
Sum 16 EPA-PAH	0,12	µg/l			ISO28540:2011	n.a | n.a
Sum NPD	0,97	µg/l			ISO28540:2011	n.a | n.a

Explanation: PQL = Practical Quantification limit. # = The analysis is performed by sub contractor.

The uncertainty is expressed at 95% confidence level. If both a relative and an absolute uncertainty argument is stated, it is the argument that represents the highest uncertainty that applies.

Intertek

Laboratory Report

Sample marking	Tank 2 - Metaller	
Sampled Date	17.mar.2014	15:30:00
Sample type	Drainwater	

Results for sample 2014-02204-005

Parameter	Results	Unit	PQL Lower Upper	Method/standard	Uncertainty Rel | Abs
Metals in seawater, ICP-MS					
Arsenic, As	<1,0	µg/l	1,0 5000	Basert på EPA200.8	15% | ±3,0
Barium, Ba	110	µg/l	10 1000000	Basert på EPA200.8	20% | ±30
Cadmium, Cd	<0,15	µg/l	0,15 5000	Basert på EPA200.8	15% | ±0,45
Nickel,Ni	<1,5	µg/l	1,5 5000	Basert på EPA200.8	20% | ±4,5
Chromium, Cr	<0,4	µg/l	0,4 5000	Basert på EPA200.8	20% | ±1,2
Copper, Cu	0,53	µg/l	0,5 5000	Basert på EPA200.8	30% | ±1,5
Iron, Fe	21	µg/l	20 400000	Basert på EPA200.8	15% | ±60
Lead, Pb	<0,25	µg/l	0,25 5000	Basert på EPA200.8	20% | ±0,75
Zinc, Zn	<4	µg/l	4 1000000	Basert på EPA200.8	25% | ±12

Explanation: PQL = Practical Quantification limit. # = The analysis is performed by sub contractor.

The uncertainty is expressed at 95% confidence level. If both a relative and an absolute uncertainty argument is stated, it is the argument that represents the highest uncertainty that applies.

Intertek

Laboratory Report

Sample marking	Tank 2 - Hg	
Sampled Date	17.mar.2014	15:30:00
Sample type	Drainwater	

Results for sample 2014-02204-006

Parameter	Results	Unit	PQL Lower Upper	Method/standard	Uncertainty Rel | Abs
Mercury in water, FIMS					
Mercury, Hg	<0,05	µg/l	0,01	Mod. NS-EN 1483	15% | ±0,01

Explanation: PQL = Practical Quantification limit. # = The analysis is performed by sub contractor.

The uncertainty is expressed at 95% confidence level. If both a relative and an absolute uncertainty argument is stated, it is the argument that represents the highest uncertainty that applies.

Intertek

Laboratory Report

Sample marking	Tank 3 - OIW	
Sampled Date	17.mar.2014	15:30:00
Sample type	Drainwater	

Results for sample 2014-02204-007

Parameter	Results	Unit	PQL Lower	PQL Upper	Method/standard	Uncertainty Rel | Abs
Oil in water, (C7-C40), GC/FID						
Oil in Water (C7-C40)	<0,5	mg/l	0,4		Mod. NS-EN ISO 9377-2 / OSPAR 2005-15	15% | ±0,2

Explanation: PQL = Practical Quantification limit. # = The analysis is performed by sub contractor.

The uncertainty is expressed at 95% confidence level. If both a relative and an absolute uncertainty argument is stated, it is the argument that represents the highest uncertainty that applies.

Intertek

Laboratory Report

Sample marking	Tank 3 - Metaller	
Sampled Date	17.mar.2014	15:30:00
Sample type	Drainwater	

Results for sample 2014-02204-008

Parameter	Results	Unit	PQL Lower Upper	Method/standard	Uncertainty Rel | Abs
Metals in seawater, ICP-MS					
Arsenic, As	<1,0	µg/l	1,0 5000	Basert på EPA200.8	15% | ±3,0
Barium, Ba	570	µg/l	10 1000000	Basert på EPA200.8	20% | ±30
Cadmium, Cd	<0,15	µg/l	0,15 5000	Basert på EPA200.8	15% | ±0,45
Nickel,Ni	<1,5	µg/l	1,5 5000	Basert på EPA200.8	20% | ±4,5
Chromium, Cr	<0,4	µg/l	0,4 5000	Basert på EPA200.8	20% | ±1,2
Copper, Cu	<0,5	µg/l	0,5 5000	Basert på EPA200.8	30% | ±1,5
Iron, Fe	49	µg/l	20 400000	Basert på EPA200.8	15% | ±60
Lead, Pb	<0,25	µg/l	0,25 5000	Basert på EPA200.8	20% | ±0,75
Zinc, Zn	<4	µg/l	4 1000000	Basert på EPA200.8	25% | ±12

Explanation: PQL = Practical Quantification limit. # = The analysis is performed by sub contractor.

The uncertainty is expressed at 95% confidence level. If both a relative and an absolute uncertainty argument is stated, it is the argument that represents the highest uncertainty that applies.

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Intertek

Laboratory Report

Sample marking	Tank 3 - Hg	
Sampled Date	17.mar.2014	15:30:00
Sample type	Drainwater	

Results for sample 2014-02204-009

Parameter	Results	Unit	PQL Lower Upper	Method/standard	Uncertainty Rel | Abs
Mercury in water, FIMS					
Mercury, Hg	0,11	µg/l	0,01	Mod. NS-EN 1483	15% | ±0,01

Explanation: PQL = Practical Quantification limit. # = The analysis is performed by sub contractor.

The uncertainty is expressed at 95% confidence level. If both a relative and an absolute uncertainty argument is stated, it is the argument that represents the highest uncertainty that applies.

Intertek

Laboratory Report

Sample marking	Tank 4 - OIW	
Sampled Date	17.mar.2014	15:30:00
Sample type	Drainwater	

Results for sample 2014-02204-010

Parameter	Results	Unit	PQL Lower	PQL Upper	Method/standard	Uncertainty Rel | Abs
Oil in water, (C7-C40), GC/FID						
Oil in Water (C7-C40)	<0,5	mg/l	0,4		Mod. NS-EN ISO 9377-2 / OSPAR 2005-15	15% | ±0,2

Explanation: PQL = Practical Quantification limit. # = The analysis is performed by sub contractor.

The uncertainty is expressed at 95% confidence level. If both a relative and an absolute uncertainty argument is stated, it is the argument that represents the highest uncertainty that applies.

Intertek

Laboratory Report

Sample marking	Tank 4 - Metaller	
Sampled Date	17.mar.2014	15:30:00
Sample type	Drainwater	

Results for sample 2014-02204-011

Parameter	Results	Unit	PQL Lower Upper	Method/standard	Uncertainty Rel | Abs
Metals in seawater, ICP-MS					
Arsenic, As	<1,0	µg/l	1,0 5000	Basert på EPA200.8	15% | ±3,0
Barium, Ba	11	µg/l	10 1000000	Basert på EPA200.8	20% | ±30
Cadmium, Cd	<0,15	µg/l	0,15 5000	Basert på EPA200.8	15% | ±0,45
Nickel,Ni	<1,5	µg/l	1,5 5000	Basert på EPA200.8	20% | ±4,5
Chromium, Cr	<0,4	µg/l	0,4 5000	Basert på EPA200.8	20% | ±1,2
Copper, Cu	<0,5	µg/l	0,5 5000	Basert på EPA200.8	30% | ±1,5
Iron, Fe	<20	µg/l	20 400000	Basert på EPA200.8	15% | ±60
Lead, Pb	<0,25	µg/l	0,25 5000	Basert på EPA200.8	20% | ±0,75
Zinc, Zn	<4	µg/l	4 1000000	Basert på EPA200.8	25% | ±12

Explanation: PQL = Practical Quantification limit. # = The analysis is performed by sub contractor.

The uncertainty is expressed at 95% confidence level. If both a relative and an absolute uncertainty argument is stated, it is the argument that represents the highest uncertainty that applies.

Intertek

Laboratory Report

Sample marking	Tank 4 - Hg	
Sampled Date	17.mar.2014	15:30:00
Sample type	Drainwater	

Results for sample 2014-02204-012

Parameter	Results	Unit	PQL Lower Upper	Method/standard	Uncertainty Rel | Abs
Mercury in water, FIMS					
Mercury, Hg	<0,05	µg/l	0,01	Mod. NS-EN 1483	15% | ±0,01

Explanation: PQL = Practical Quantification limit. # = The analysis is performed by sub contractor.

The uncertainty is expressed at 95% confidence level. If both a relative and an absolute uncertainty argument is stated, it is the argument that represents the highest uncertainty that applies.