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Uptake of uranium in Atlantic salmon *(Salmo salar)* – aqueous exposure

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Chemistry and Biotechnology – Technology (Civil engineer) - Master

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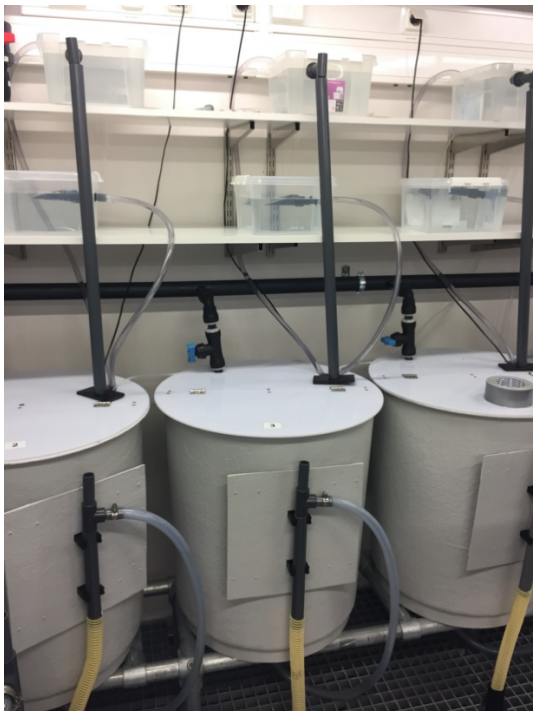

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Birgith Øverby Terum, Msc.

Statement of declaration

The thesis is the final part of the civil engineer study in chemistry and biotechnology, specialization in Environmental Chemistry, at the Norwegian University of Life Sciences (NMBU), Aas, Norway. The thesis is a part of a larger Ph.D study conducted by Shane Scheibener. He had the full responsibility for the experiment and lead the work completely. I participated in the set-up of exposure tanks, dissection of the exposed fish tissues (gills, kidney, liver, stomach, brain, muscle and bone) two days in June and two days in August), and some day-to-day work like feeding, water exchanges, water sampling, onsite water fractionation and daily measurements of water quality. I participated in the pre-treatments of the tissue samples, i.e. freeze-drying, weighing and preparing for digestion. I took part in the analysis on ICP-MS of both water and tissue samples.

List of acronyms

Bq: Becquerel

EtOH: ethanol

^{o}C: degrees of Celsius

Ca: calcium

CO_2: carbon dioxide

CRM: certified reference material

DO: dissolved oxygen

DOC: dissolved organic carbon

DOM: dissolved organic material

DU: depleted uranium

dw: dry weight

F: fluoride

fw: fresh weight

g: gram

HCl: hydrochloric acid

He: helium

HNO_3: nitric acid

ICP-MS: inductive coupled plasma – mass spectrometry

i.e.: *id est* (in other words)

K: potassium

kDa: kilo Dalton

L: litre

LMM: Low Molecular Mass

LOD: level of detection

LOQ: level of quantification

Mg: magnesium

N: number

Na: sodium

NH_3: ammonia

NH_4^+: ammonium

NO_3^-: nitrate

NORM: naturally occurring radioactive material

OECD: Organization for Economic Cooperation and Development

P: Phosphorus

Pb: lead

pH: the negative log of the concentration of the hydrogen cation ($pH = -\log_{10}[H^+]$)

PO_4^{3-}: phosphate

Pu: plutonium

Ra: radium

Rn: radon

RO-water: reverse osmosis water

RSD: relative standard deviation

S: sulphur

SO_4^{2-}: sulphate

Th: thorium

TOC: Total organic carbon

U: uranium

µ: micro

UF: Ultrafiltration

UP: ultrapure

US- EPA: United States Environmental Protection Agency

vv: volume volume (concentration)

ww: wet weight

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Abstract

The MSc. thesis assess the ecotoxic effects of uranium in freshwater ecosystems, by analysing the possible uptake-routes of U in fish using juvenile Atlantic salmon (*Salmo salar)* as a model organism. Bioconcentration factor (BCF), uptake-rates and organ distribution of accumulation were used to determine the potential U uptake.

Objectives: (1) There is an uptake of uranium in the fish directly from water. (2) The concentration of uranium in gills is higher than in stomach at steady state in aqueous exposure. (3) Uptake-rates in gills and skin, compared to muscle, kidney and liver are higher;

Atlantic salmon (*Salmo salar*) (n=50) were exposed to waterborne uranium in moderate hard US-EPA water for 28 days. A stable pH (6.7) and water conditions were maintained in the exposure to keep the speciation of U constant. Water samples were collected every day and water fractionation-samples were collected three times during the exposure (day 2, 17 and 28). Fish were sampled five times during exposure using the EMERGE protocol (Rosseland et al., 2001). The bioaccumulation of U in the different tissues was quantified. Elemental concentrations were quantified using Agilent Technologies 8800- QQQ ICP-MS. The precision and accuracy were considered based upon the analysis of certified reference materials, estimated relative standard deviation (RSD), and quantification and detection limits.

The general water quality was not significantly different between the control- and 50 µg U/L-treatment. The average concentration in the nominal 50µg/L experiment was 55 ± 22 µg U/L (n=32) and 0.027 ± 0.038 µg U/L (n=38) in the control exposure waters. The low molecular mass (LMM) of uranium was present as 19 % of total U concentration. The assumed bioavailable (U-cations) concentration was on average 1.13 ± 1.74 µg U/L of total U concentration. U spikes, water exchanges and rapid sorption of bioavailable U to dissolved organic matter (DOM) in the water did likely cause a dynamic change in fractionation of U.

The accumulation of uranium was significant in all of the organs analysed after 28 days of exposure. It was no significant U uptake in the organs from the control-exposure. The highest U concentration and uptake-rate were quantified in the gills (5.9 ± 0.9 µg/g tissue ww and 0.22-0.24 µg U/g tissue/day). The U concentrations were as follows: gills>stomach w/content >skin>kidney>muscle>liver. The uptake-rates were significantly higher in the organs with

direct contact with the contaminated water, compared to the internal organs analysed (muscle, kidney and liver). The apparent BCF in liver was 0.52 L/mg (<0.45 µm-filtered U conc.). The BCF in stomach (w/content) was 18.5 L/mg (particulate U conc.). The other organs did not reach steady state concentrations within 28 days of exposure.

The accumulated concentrations in internal organs (muscle, kidney and liver) documented U being transported in the blood. Uptake of U through skin and stomach were not excluded in aqueous exposure of U to Atlantic salmon. Results demonstrate that even longer exposure are needed to identify steady state.

Key Words: Uranium, Atlantic Salmon (*Salmo salar*), Uptake-rate, U-accumulation, U-concentration, U-fractions, bio concentration factor

Sammendrag

Masteroppgaven omhandler økotoksikologiske effekter av uran i økosystemer i ferskvann, ved å analysere de mulige opptaksveiene av uran i fisk ved å benytte ung (3 mnd gml.) Atlantisk laks (*Salmo salar*) som modellorganisme. Biokonsentrasjonsfaktor (BCF), opptakshastighet og konsentrasjonsfordeling i organer ble benyttet for å bestemme det potensielle opptaket av uran.

Hypoteser: (1) Uran blir tatt opp i fisk direkte fra vann. (2) Konsentrasjonen av uran i gjeller er høyere enn i mage ved likevekt ved eksponering i vann. (3) Opptakshastigheten i gjeller og skinn er større, sammenlignet med muskel, nyre og lever.

Atlantisk laks (n=50) ble eksponerer for vannbåret uran i moderat hardt US-EPA vann i 28 dager. En stabil pH (6.7) og stabile betingelser for vannet medførte at U spesieringen var konstant. Vannprøver ble samlet hver dag og fraksjonering av vann ble utført tre ganger underveis i eksponeringen (dag 2, 17 og 28). Det ble tatt prøver av fiskene fem ganger i løpet av eksponeringen. Selve prøvetakingen fulgte EMERGE protokollen (Rosseland et al., 2001). Bioakkumuleringen av U ble målt de forskjellige vevene. Konsentrasjoner av målte grunnstoff ble kvantifisert ved bruk av Agilent Technologies 8800- QQQ ICP-MS. Presisjonen og nøyaktigheten ble bestemt basert på analysen av de sertifiserte referansematerialene, de estimerte relative standard avvikene (RSD), og kvantifikasjons- og deteksjonsgrense.

Den generelle vannkvaliteten var ikke signifikant forskjellig mellom kontrollen og 50 µg/l uran-eksponeringen. Den gjennomsnittlige konsentrasjonen av uran i den nominelle 50 µg/l-eksponeringen var 55 ± 22 µg U/L (n=32) og 0.027 ± 0.038 µg U/L (n=38) i kontrollen. Den lavmolekylære fraksjonen av uran tilsvarte 19 % av den totale konsentrasjonen. Den antatte biotilgjengelige (U kation) konsentrasjonen var i gjennomsnitt 1.13 ± 1.74 µg U/L av den totale uran-konsentrasjonen i vannet. Tilsetning av U, jevnlige bytter av eksponeringsvann og rask binding av biotilgjengelig uran til løst organisk materiale (DOM) skapte en dynamisk forandring i fraksjoneringen av U.

Etter 28 dager var akkumuleringen av uran signifikant i alle de analyserte organene. Det var ikke et signifikant opptak av U i kontrollene. Den største U konsentrasjonen og opptakshastigheten ble kvantifisert i gjellene (5.9 ± 0.9 µg/g gjelle ww og 0.22-0.24 µg U/g

gjelle/dag). Konsentrasjonene av uran var i synkende rekkefølge: gjeller, mage m/innhold, skinn, nyre, muskel og lever. Opptakshastighetene var signifikant høyere for de organene med direkte kontakt med det kontaminerte vannet, sammenlignet med de indre organene som ble analysert (muskel, nyre og lever). Den tilsynelatende BCF i lever var 0.52 L/mg (<0.45 µm-filtrerte U kons.). BCF i mage m/innhold var 18.5 L/mg (partikulær kons.). De andre organene oppnådde ikke likevekt i løpet av de 28 dagene med U-eksponering.

De akkumulerte konsentrasjonene i de indre organene (muskel, nyre og lever) dokumenterte at uran ble transportert i blodet. Opptak via skinn eller mage ble ikke ekskludert i Atlantisk laks gjennom vanneksponering av U. Resultatene viste at lengre eksponeringstid er nødvendig for å kunne oppnå likevekt.

Nøkkelord: Uran, Atlantisk laks (*Salmo salar*), opptakshastighet, uran-akkumulering urankonsentrasjon, U-fraksjoner, biokonsentrasjonsfaktor

1.Introduction

1.1 Uranium

1.1.1 Radioactivity

Natural uranium consists of three isotopes ^{238}U, ^{235}U and ^{234}U (Barillet et al., 2007). There is 99.2745 % of ^{238}U, 0.7200 % of ^{235}U and 0.0055 % ^{234}U (Choppin et al., 2002a). The half-life of ^{238}U is about the same as the age of the Earth (4.5×10^9 years), which states that this isotope is not very radioactive (Bleise et al., 2003). The specific activity of ^{238}U is 12.4 MBq/kg and 25.4 MBq/kg of natural uranium (Choppin et al., 2002a). ^{238}U is the parent isotope of several more radiotoxic elements like thorium (Th), plutonium (Pu), radium (Ra) and radon (Rn). All of the daughter nuclides have shorter half-life than the ^{238}U isotope. The final product in the U decay chain is lead (^{206}Pb), which is stable (Bourdon et al., 2003).

^{235}U (0.72 % of natural uranium) is fissile, i.e. capable to undergo fission, which is the production of electricity occurring in a nuclear reactor. Before U may be used as nuclear fuel, it has to undergo an enrichment process where the ^{235}U percentage is increased to about 3 %. Before the enrichment process starts, U is required to be in gaseous form and is then converted to a fluoride. After the process is finished, the uranium fluoride (UF_6) is separated to enriched U and low enriched U, also known as depleted uranium (Choppin et al., 2002b)

1.1.2 Sources of U

Several sources have contributed to concentrations of radioactivity in the environment and naturally occurring radioactive materials (NORMs). Weathering of U- and Th- containing minerals is a natural event and nuclear weapon and fuel cycle (U mining), and oil and gas-industries are all anthropogenic or man-made activities (Salbu et al., 2015). The anthropogenic nuclear weapon and fuel cycles are the main sources of U contamination to the environment (Goulet et al., 2012). Production and testing of nuclear weapons generate a lot of radioactive waste. Atmospheric testing of nuclear weapons is the main source of plutonium in the environment, which is known to be a highly radioactive element (Finch et al., 1999).

The nuclear fuel cycle is divided into three steps, the front-end, the nuclear power station and the back-end part. The front-end includes mining, milling and enrichment, and the back-end includes radioactive waste treatments (Choppin et al., 2002). Mining, milling and refining

generates the greatest volumes of radioactive waste. The spent nuclear fuel is the largest source of radioactivity (Finch et al., 1999).

1.1.3 Depleted uranium

U and DU have the same behaviour in the body, but DU is about 60 % less radioactive (Bleise et al., 2003). The concentration of ^{235}U in depleted uranium is 0.2-0.3 %, compared to about 0.7 % in natural uranium (UNEP, 2001). Due to the low radioactivity, DU is commonly used to analyse mechanisms and model uptake of natural uranium (Song et al., 2014).

DU is a biproduct of the pre-treatments of natural uranium (Lind et al., 2020). In several countries, DU is used in military purposes as armour plating and ammunition (Bleise et al., 2003). To characterize U as DU, the concentrations of ^{235}U and ^{234}U are reduced relative to the concentration of ^{238}U. The weight of ^{235}U has to be 0.2 % of the total mass of DU, and the weight of ^{238}U has to be 99.8 % (WHO, 2001). The atom-ratio between the isotopes ($^{235}U/^{238}U$) in DU is reported to be 0.002 (Lind et al., 2020).

1.2. Uranium in water

1.2.1 Naturally concentrations of U

The concentration of U in water is dependent on the geological material surrounding the water source (WHO, 2001). In 2005, aqueous concentrations (lakes, rivers) of total uranium were determined in several countries (Australia, Canada, Kyrgyzstan and Kazakhstan) with large U reservoirs, to detect possible U contaminations from the mining activities. Natural background U concentration is quantified as <1 µg/L. In Australia all concentrations were below natural background (n= 525). About 75 % (n= 68 303) of the quantified U concentrations in Canada were below natural background concentration. The remaining 25 % ranged up to 1350 µg U/L, as a result the clean-up technology was improved, and the concentrations got closer to natural background in 2009. In central Asia (Kyrgyzstan and Kazakhstan), the U concentrations in the rivers reached maximums of 3.1 µg/L (n= 14) and 41 µg/L (n= 160) (Goulet et al., 2012). Pit lakes (U mining sites) in central Asia (Kyrgyzstan, Kazakhstan and Tajikistan) had U concentrations ranging from 7.8 µg/L to 3 mg/L (Lind et al., 2013; Salbu et al., 2013; Skipperud et al., 2013b; Strømman et al., 2013).

In 1996, the Norwegian Institute of Water Research (NIVA) conducted a study determining the concentrations of different elements in Norwegian lakes (n=475). The concentration of U

was in the range of 0.004-2.22 µg/l (Skjelkvåle et al., 1996). Several Norwegian groundwaters (n=426) were analysed to detect the concentration of different elements (Be, Th, U, Cd, Hg etc.) in 2000. The water was sampled from boreholes in Norwegian crystalline bedrocks. The concentration of U in 18 % of the samples exceeded the American maximum admissible concentration of 20 µg/l. The median was 2.5 µg U/l and the highest quantified concentration was at 750 µg U/l (Frengstad et al., 2000).

1.2.2 Speciation of U in water

The natural low concentration of uranium in rivers and lakes originates mainly from the erosion of rocks and minerals (Komperød et al., 2015). In water, the metal exists as U(IV) or U(VI), and the form is dependent on the redox potential in the water. The U(IV)-species are almost insoluble and normally found in sediments, while U(VI)- species are in general mobile and soluble (Goulet et al., 2012). In a reducing environment, U(VI) is reduced to U(IV), likely due to microbial involvement (Windom et al., 2000).

1.2.3 Factors affecting the speciation of U in water

The bioavailability of a metal and the further toxicity in natural water is highly dependent on the physio-chemical form of the metal (speciation) (Franklin et al., 2000). The speciation of U in water depends on the pH, dissolved organic material and dissolved phosphorus (Goulet et al., 2012).

The speciation of U is directly linked to pH. The uranyl-ion (UO_2^{2+}) may form complexes with either hydroxy-(OH-) or carbonates- group(s) (CO_3^{2-}) when the pH >5, in oxic freshwater (Goulet et al., 2012). At pH 6.7, the primary low molecular mass U species are: UO_2^{2+}, $UO_2CO_3^0$, $UO_2(OH)_2^0$ and UO_2OH^+, as well as other complexes with hydroxy- or carbonate-ligands (figure 1.1). pH 6.7 is commonly observed in Norwegian lakes (Solheim et al., 2018).

Dissolved organic material (DOM), or dissolved organic carbon (DOC), are strong ligands, which may bind to dissolved uranium in freshwater (Trenfield et al. 2011). DOM has a negative charged surface, which make cations in the water easily sorb to the surface and cause an in-direct inhibition of accumulation onto other surfaces in the same environment (mucus, fish gills etc.) (Rosseland, 2000). Higher concentrations of DOM and DOC may decrease the concentration of the uranyl-ions (UO_2^{2+}), and increase the concentrations of colloidal- and

particulate U. DOM forms stable complexes with the uranyl-ion in freshwater **(figure 1.1**, Goulet et al., 2012), and particulate U in freshwater sediments is affected by deposition of DOM (McManus et al., 2006; Chappaz et al., 2010). The complexation of organic matter and metals increases with pH and the solubility of metals decreases with pH, which both affect the formation of metal-ligands in freshwater with a pH closer to neutral (Franklin et al., 2000).

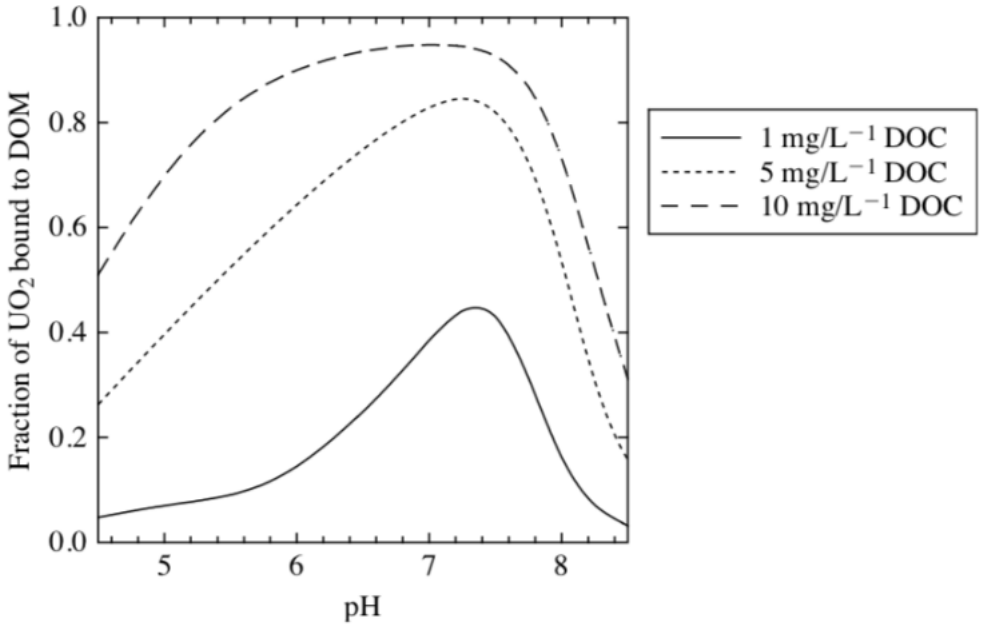

Figure 1.1: The graphs indicate the fraction of total UO_2 bound to DOC at different concentration affected by pH (Goulet et al., 2012).

Phosphorus (P), or phosphate, at high concentrations in surface waters has the possibility to form complexes with U and decrease the fraction of bioavailable U species. Uranium may form complexes with phosphate ions if the concentrations are >0.1 µg/l, which is not assumed to occur in natural freshwater (Goulet et al., 2012).

The distribution of U species changes depending on available ligands. Uranium may form complexes with sulphate or fluoride (>1 % of present species) if the pH is low (<6.2). The uranyl ion is often referred to as the “free U ion”, but it is in fact coordinated by water molecules (Goulet et al., 2012).

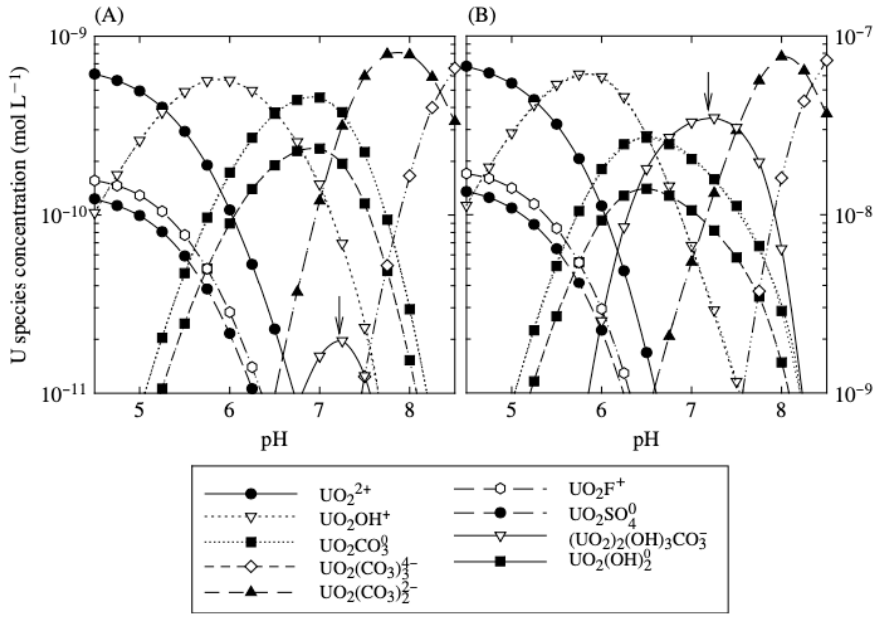

Figure 1.2: Speciation of LMM-U linked to pH. Uranium concentration in A) is 1nmol/L an B) is 110 nmol/L (Goulet et al., 2012).

1.2.4 Fractionation in water

The physio-chemical properties define the radionuclide species, for instance oxidation state, charge properties and valence, density, structure, degree of complexation and nominal molecular mass (Salbu, 2007). The U compounds can be divided into different fractions. The definitions by size are that particles have a diameter larger than 0.45µm, colloids or pseudocolloids have a diameter 1 nm-0.45 µm, and LMM-species or low molecular mass species have a diameter less than 1 nm (Salbu, 2007). Colloidal masses are larger than 10kDa and LMM species are less than 10kDa (Teien, pers comm). The U in pit lakes in central Asia were mainly present as LMM-species (DellaSala and Goldstein, 2017; Skipperud et al., 2013b)

In a similar study analysing U chemotoxicity was the predominantly part LMM-species, and about 30 % U colloids (Teien et al., 2014). The LMM- species are believed to be the most mobile and bioavailable fraction, i.e. can be transported by active uptake (across cell membranes). Particles and colloids are considered to be inert biologically (**figure 1.3**) (Salbu, 2007). The colloids are considered to be mobile in water. The particles are believed to be the most immobile fraction, which precipitates in water and is likely found in the sediments (DellaSala and Goldstein, 2017). All the fractions consists of different U species (Goulet et al., 2012).

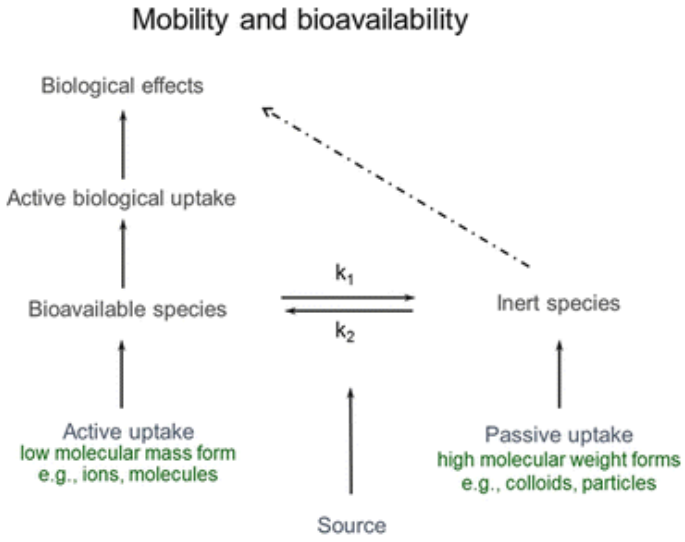

Figure 1.3: Overview of fractions (Salbu et al., 2015)

1.3 Uptake of uranium in fish

1.3.1 Bioavailability of U

In aqueous exposure, U(VI) is more favourable than U(IV) to be taken up in the fish. However, U(IV) may sorb to the particles in the sediments and be eaten by fish (Goulet et al., 2012). The uranyl-ion has a positive charge, which makes it likely to bind to the negatively charged mucus and surface of gills like other cations (ex.: Al^{3+}) (Rosseland, 2000). Other LMM-U is less bioavailable due to a less positive, neutral or negative charge (**figure 1.2**) (Goulet et al., 2012).

The pH may affect the accumulation of U in two ways: by changing U speciation or the H^+-concentration. Franklin et al. (2000) studied the toxicity of U in algae at pH 5.7 and 6.5. The results revealed different inhibition of growth in the two exposures. pH 6.5 was identified as the most toxic pH, and because the U speciation were fairly similar in the two exposures, the U toxicity was linked to the H^+-concentration where the H^+-ion inhibited the uptake of U at the lower pH (Franklin et al., 2000).

The uranyl-ion (UO_2^{2+}) is considered to be the most bioavailable specie of U (Goulet et al., 2012). It has been assumed that high concentrations of phosphate in water reduces the bioavailability of U. However, the phosphate concentration is predicted to be well above 60 µg/L in order to make an effect of the toxicity of U (Goulet et al., 2012) .

1.3.1. Uranium toxicity

Natural and depleted uranium are considered to have low radiotoxicity (Giblin et al., 2015). U has a larger potential as chemotoxic than radiotoxic (Mathews et al. 2009), because of the low specific activity (Simon and Garnier-Laplace, 2005)

The activity of the free hydrated metal-ion is considered to affect the metal toxicity in water. The total metal concentration is less relevant to estimate the toxicity (Franklin et al., 2000). There is little information about the U toxicity to fish, but U is considered to be one of the less toxic metals. The extent of chronic effects of U exposure have limited information, and most of the mechanisms are probably unknown (Goulet et al., 2012).

1.3.2 Organ distribution

Bioaccumulation of U in fish is documented in previous studies (Barillet et al., 2007; Lerebours et al., 2009; Song et al., 2014; Simon et al., 2019). The metal does not biomagnify in the food chain (Goulet et al., 2012). Within fish tissue, U generally accumulates in mineralized tissues like bone and scales. Smaller concentrations may be detected in kidney and liver. Detectable concentrations are likely to be determined in the gill filaments, skin and muscle. Concentrations of U have been detected in the brain, which makes U one of few metals which pass the blood-brain barrier (Barillet et al., 2007; Goulet et al., 2012; Song et al., 2014).

The World Health Organization (WHO) states that U(VI)-compounds may become adsorbed through the skin in humans (WHO, 2001). There is little information about U sorption to fish skin, or skin as a possible uptake-route. In Barillet´s study (2007), the highest concentrations of U were detected in the liver and gills, when the same organs as listed above were analysed. U accumulates heterogeneously in fish, which is likely explained by the organs different physiological roles in uptake and transport of U (Barillet et al., 2007; Lerebours et al., 2009). U is often bound to bicarbonate when transported in the blood. The kidney filters the blood, and this may cause higher concentrations of U in this tissue (Goulet et al., 2012). The kidney is considered as the main target organ in humans (WHO, 2001). The U accumulation depends further on the concentration of bioavailable U, typically reactive LMM U species, and the total U concentration is not directly related to the accumulation (Song et al., 2014).

A mass balance approach was conducted to demonstrate in which tissues U generally accumulates. This model predicts about 20 % of the U uptake to accumulate in the bones, 18 % in remaining hard tissues, 2.8 % in gonads, 0.6 % in kidney, 1.3 % in liver, 43 % in muscle and 15 % in remaining soft tissues (Yankovich, 2009). This model has not been validated in the field (Goulet et al., 2012)

In the same pit lakes as previously listed (Kazakhstan, Tajikistan and Kyrgyzstan), U concentration in fish were studied. The results showed U accumulation in gills, liver, kidney, (bone)[1] and muscle. The U distribution varied between the fish species, but generally the highest accumulation was quantified in the gills. At similar U concentrations in the water in Kazakhstan and Tajikistan, the highest accumulations of U were in the liver and gills. The pH at the two sites were 8.5 and 8, respectively (**table A.1**, Appendix 1) (Salbu et al., 2013; Strømman et al., 2013; Skipperud et al., 2013b; Lind et al., 2013).

1.3.2.1 Uptake through the gills

Few studies have analysed the biokinetics of uptake of uranium through the gills (Goulet et al., 2012; Lerebours et al., 2009; Simon et al., 2019). Ions like calcium and magnesium have the same chemical potential as U. It is likely that uranium can be taken up through the same transport mechanisms as the active uptake of Ca and Mg in the gill membrane (Lerebours et al., 2009; Giblin et al., 2015).

Lerebours et al. (2009) completed an experiment with U uptake in zebrafish. The total concentrations of U in the water, as well as in organs, were quantified. Concentrations in the gills and liver were not significantly different throughout the exposure (4000 ng U/g dw). The concentration in skeletal muscles was quantified to be about 700 ng U/g dw (Goulet et al., 2012; Lerebours et al., 2009). The steady-state of U uptake was not reached in this experiment (Lerebours et al., 2009). The uptake appeared to increase asymptotically over the 28 days of exposure, by following a single-compartment first order kinetics (Goulet et al., 2012).

Uranium accumulates in fish gills, and the accumulation correlates positively with the concentration of U in the water. The uptake is dependent on the pH; in the range of 5.5-6.0 is the accumulation to the gill high and in the range 7.3-7.7 is the accumulation lower (Giblin et al., 2015). The uptake through the gills may decrease if the uranyl-ion is blocked by the

[1] Bone was not included in the analysed tissues in this study.

protective mucus-layer on the gills surface. This blocking-effect has been revealed for other divalent metals (Barillet et al., 2007; Lerebours et al., 2009).

Lead (Pb) has similar traits as U (Nieboer and Richardson 1980). A study conducted by Hodson (1978), compared uptake routes and accumulation of lead in different organs for rainbow trout (*S. Gairdneri)*. The main uptake was from water and through the gills, and the central accumulation was in the bones, gills and kidney. Uptake from dietary Pb-exposure was low compared to the aqueous exposure, and was not above control levels (Hodson et al., 1978).

1.3.2.2 Uptake through the gut

Few reports describe how U is taken up through the gut in fish from contaminated food. U is a reactive trace element, which may sorb to the surface of particles and colloids. The fish can eat these objects, which may cause a detectable U concentration in the stomach. Some data indicate that fish living close to sediments (benthic) have an uptake of U through the stomach (Goulet et al., 2012).

Bleise et al. (2003) referred to previous studies conducted by Harley et al. (1999) analysing the uptake of U in animals. The uptake from the ingested DU contaminated food was about 2-5 % from the intestines to the blood. The rest of the U in the food was assumed to just pass the intestines and not be taken up in the body. The major concentration (90 %) of U in the blood was predicted to be excreted though the kidneys, within a week. The last 10 % was assumed to accumulate in the organs, whereabout 15 % was predicted to accumulate in the bones (Bleise et al., 2003). The same trends for uptake through the gut in animals may be seen in fish too, but this is not known. In humans were the uptake-percentage of U from contaminated food about 2 % (WHO, 2001).

Simon et al. (2019) conducted a similar study analysing the uptake-routes in waterborne (20 µg/L U) and dietary (10.7 µg/L, ^{233}U (radioisotope) exposure of U on zebrafish (*D. rerio*). The aqueous exposure was either performed alone or in combination with the dietary. The timespan was 5 or 20 days. The pH was 6.5 ± 0.7. The total U concentration in the water was in the range of 21-22 µg/L. The intestines had the highest U accumulation after 20 days of waterborne exposure compared to the other organs analysed (gill epithelium, liver, kidneys,

gonads, and intestine). The concentrations in the intestines (10-15 µg/g tissue fw) were about thirty times higher than the quantified concentrations in the gills after 20 days.

The U concentration provided by the water was two times higher than the concentration provided by the diet. Simon et al. included the aspect of absorption of waterborne U to the uncontaminated food, but the food was consumed by the fish fast (1-5s) which reduced the contact time. The present decrease of U in both exposures were not significantly different. The measured diet-borne transfer of the radioactive U isotope (^{233}U) was quantified as low, which reduced the weight of intestine contamination via food consumption (Simon et al., 2019).

In another study, Crayfish (*O. Limosus*) were fed with U contaminated bivalves. The trophic transfer order differed between individuals (1-13 %). The U accumulated in the stomach and digestive gland and reached concentrations of about 12 µg/g fw and 18 µg/g fw respectively (Simon and Garnier-Laplace, 2005).

1.3.2.3 BCF of uranium in fish

The bioconcentration factor is the transfer from water to fish (Lind et al., 2013). The BCF may only be calculated when the uptake of U reaches a steady state, i.e. the uptake concentration in the organ is the same as the concentration eliminated. The uptake of U over time will likely slow down (Bleise et al., 2003).

$$\mathbf{1}.\ BCF = \frac{U\ concentration\ (fish)\frac{\mu g}{kg}dw}{U\ concentration\ (water)\mu g/L} = L/kg$$

(Skipperud et al., 2013a)

There are few calculated BCFs describing the uptake of U in fish. According to Goulet et al., it is quantified values from 0.001 to 0.149 L/kg. The concentrations stem from several studies (Barillet et al., 2007 etc.) with different water chemistry and various fish species. Another uncertainty is the fact that U is usually measured in some tissues and not the whole body-concentration (Goulet et al., 2012).

The bioconcentration factors were quantified in tissues (liver, gill, muscle and kidney) in Kazakhstan, Tajikistan and Kyrgyzstan (**table 1.1**). The BCF varied between tissues, but

generally the highest BCF was quantified in the gills. At similar U concentrations in the water in Kazakhstan and Tajikistan, the highest BCFs were quantified in the liver and gills (Salbu et al., 2013; Strømman et al., 2013; Lind et al., 2013).

Table 1.1: Overview of quantified BCFs at Kazakhstan, Tajikistan and Kyrgyzstan.

Site	**U conc. in water**	**pH**	**TOC** (mg/L)	**BCF**	**Reference**
Kazakhstan	1.3 mg/L	8.5	1.76	Liver: 2.5 L/kg (dw)	(Salbu et al., 2013; Strømman et al., 2013).
				Gill: 2.9 L/kg (dw)	
				Muscle: 0.11 L/kg (dw)	
Tajikistan	1.4 mg/L	8	2.23	Liver: 5.6 L/kg (ww)	(Strømman et al., 2013)
				Gill: 3.6 L/kg (ww)	
				Muscle: 0.12 L/kg (ww)	
				Kidney: 5.2 L/kg (ww)	
Kyrgyzstan	41 µg/L	7.6-8.7	1.48	Liver: 0.25-1.6 L/kg (ww)	(Lind et al. 2013)
				Gill: 0.63-1.9 L/kg (ww)	
				Muscle: 0.043-0.10 L/kg (ww)	

1.4 Biotic factors affecting the uptake of U in fish

1.4.1 Biodilution

A potential growth during exposure may cause a biodilution of the U concentration in the fish. The biodilution factor ought to be low to be able to reach a steady state in U uptake (Teien, pers comm).

1.5 Atlantic salmon (*Salmo salar*)

The average fertile salmon (about one year old) weighs between 1-3 kg. The salmon is an anadromous specie, which has the ability to live in both rivers and in the ocean. The fish spawns in freshwater and lives most of its life in the ocean (Hansen, 2000). Atlantic salmon (*S. Salar*) is one of the most sensitive fish species, which makes the salmon a favoured specie to use in an ecotoxic study (Poléo et al., 1997). The mucus works as an immune system for the fish, by covering external surfaces when the concentration of a pollutant reaches toxic levels for the fish (Barillet et al., 2007; Rosseland, 2000; Teien, pers comm) . Rapid changes in the ecosystem, for instance spring floods, may be critical to the juvenile salmon and cause fish death (Rosseland, 2000).

Atlantic salmon is a central fish species in Norway, with both cultural and economic aspects, which makes it important to maintain the fish health by determining possible pollutants in studies. Norway is the world's leading producer of salmon, with more than half of the world's production ("Laksefakta", Seafood Norway).

1.6 Objectives

When identifying the uptake of U in fish, the uptake-rate and the bioconcentration factor (BCF) are important factors to determine. These factors may be used to develop models to assess the ecotoxic effects of uranium in freshwater ecosystems.

Three hypotheses were tested in this study:
(1) There is an uptake of uranium in the fish directly from water;
(2) The concentrations of uranium in gills is higher than in stomach at steady state in aqueous exposure;
(3) Uptake-rates in gills and skin, compared to muscle, kidney and liver are higher;

The main goal of the experiment was to identify the uptake-rates of U in fish using juvenile Atlantic salmon (*Salmo salar)* as a model organism.

2.Method

2.1 Fish holding conditions

This master thesis was a part of a Ph.D. study, which compared uptake and depuration of uranium from waterborne U and U contaminated food. The MSc. part of the experiment focused on how fast U was taken up from waterborne exposure and the following distribution of U between tissues. Due to the extent of the experiment, only one treatment (50 µg U/L aqueous exposure) was evaluated and compared to the control in this thesis. The entire experiment followed OECD guideline 305 for bioaccumulation in fish (OECD/OCDE, 2012) for a period of 28 days. The experiment was approved by the Norwegian Animal Research Authority (FOTS ID: 19370)

2.1.1 Atlantic salmon

Atlantic salmon (*Salmo salar)* juveniles from the fish laboratory of Norwegian University of Life Science (NMBU) were used in the experiment. The eggs were obtained from Aquagen AS (Trondheim, Norway). The salmon were fed about 1-month post-hatch (before exposure). The feed was a yeasted-based pellet that met the required nutrients for the fish. This feed had low concentrations of U (0.055 ± 0.001 µg/g feed). The fish were maintained in RAS (recirculating aquaculture system) lab water during early development life-stages.

One week prior the exposure start, 50 fish (3-month-old, average weight: 1.2 g, average length: 4.8 cm) were transferred from a batch holding tank to exposure vessels and kept in US-EPA moderately hard water for acclimatization before the start of the exposure. Moderately hard water was produced from deionized water in batch-tanks (800 L) using standard recipe (table 3.3) and aerated for 24 hours prior to use.

The US-EPA moderate hard water was modified to reach a pH at 6.7 instead of 7.4-7.8 to ensure a larger fraction of the uranyl-ions to be present in the water (Goulet et al., 2012, **figure 1.2**). The concentration of $NaHCO_3$ (sodium bicarbonate) which originally was set for moderate hard water was replaced with the concentration set for soft water. The $NaHCO_3$ was the main salt affecting the pH, because the increased concentration of carbonate ions (CO_3^{2-}) in the water increased the pH. The concentration of uranyl-ions may have been affected by the excess carbonate-ions, due to increased sorption. Sodium chloride (NaCl) was added to reach the listed concentration of sodium in the water, which increased the concentration of chlorine

(Cl) in the water. The rest of the salts added, followed the set standards for US-EPA moderate hard water.

All of the water used during acclimatization and during the experiment was adjusted to pH 6.7 and kept at 15 ^{0}C. U exposure water was made using preconditioned moderate hard water, which was transferred to a separate tank (220L). U was added from a stock as uranyl hexahydrate ($UO_2(NO_3)^2$ x 6 H_2O) (Sigma Aldrich) to a final concentration of 50 µg U/L. The chronic low concentration of U in this experiment provided a constant exposure, which was not assumed to cause toxic conditions and further affect the uptake of U in fish. The U contaminated water was stored 48 hours before being transferred to the fish tank, to ensure stable water quality.

The fish were fed twice a day at 2 % bodyweight during acclimatization and throughout the entire course of experiment. The fish needed to be fed during the 28 days of exposure, as exposure without feed can only continue for some few days.

2.2 Exposure system

2.2.1 Design

Exposure tanks comprised a recirculating flow-through design and each exposure (control and 50 µg U/L) was performed in duplicates. Atlantic salmon juveniles (n=50) were placed in duplicate 25 L-tanks for both exposures (control and 50 µg U/L). Tanks containing fish were connected to a header tank (4 x silicon tubes, 5 mm inner diameter), which provided a continuous water flow (**figure 2.1**).

Overflow from the fish tank entered the 100 L tank, via a CO_2-stripper, to be recirculated. The CO_2-stripper was conducted by PVC (polyvinyl chloride) tubes filled with high surface-area plastic inserts. Freshly aerated water was provided to the header tank by a submersible water pump from a 100 L tank below the fish tank. The design of this system provided constant waterflow at a stable water level to the fish with oxygen saturated water with low CO_2. Each tank had a lid to keep the evaporation-rate as low as possible.

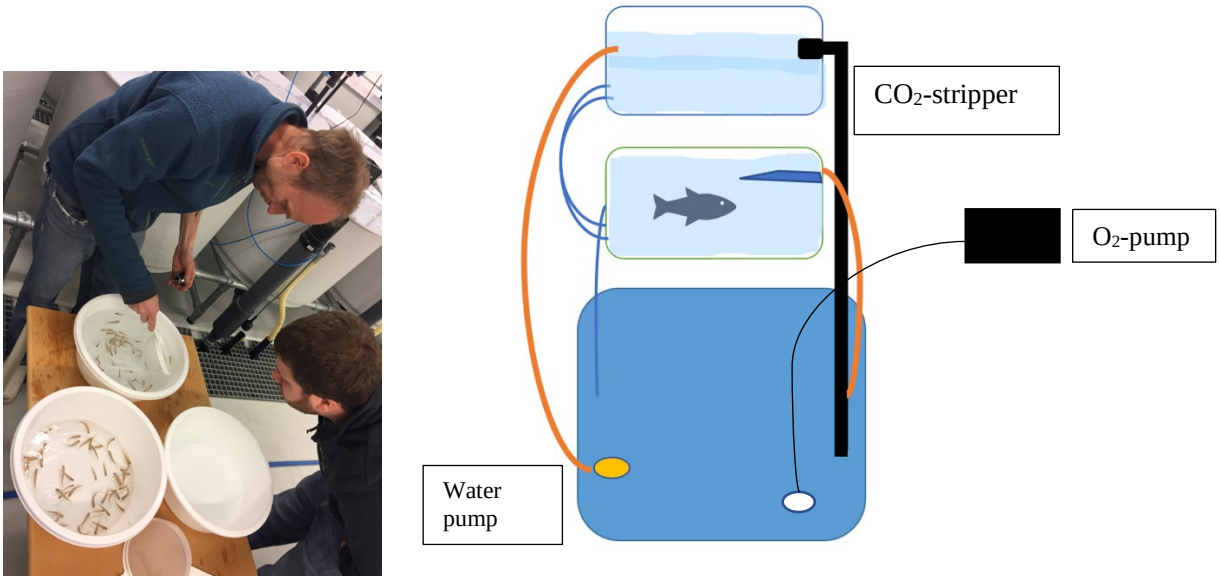

Figure 2.1: Fish (n=50) were transferred from the breeding-tank to a separate exposure- tank. The tanks were connected by tubes to maintain a continuous waterflow and a stable level of water in the exposure tank.

2.2.2 Water quality

It was important to minimize possible stress factors other than U. One goal was to ensure optimal water quality for the fish during the experiment. Temperature, pH, conductivity, CO_2-level, dissolved oxygen, NH_3/NH_4^+-concentration were then measured throughout the course of the experiment. The pH, conductivity and O_2-saturation were measured using WTW- Multi 340i and - Multi 3420. A climate-controlled room maintained constant temperature at 15^0C. The CO_2-level was measured by Oxyguard CO_2 analyser and the NH_4-level was measured by a Merck spectrophotometer.

Introduction of feed into the system increased the concentrations of ammonium (NH_4^+) by excretion from fish. Ammonium can be transformed to ammonia (NH_3) in water, which may be toxic to fish at high concentrations. To ensure low concentrations of NH_3, the concentration of NH_4 was quantified regularly. It was assumed that the feed contributed to increase the concentrations of DOM in the water, which might further increase sorption of U to DOM colloids and particles. The exposure water was changed about two times per week to decrease the concentration of DOC. All water was removed from the tanks (except the fish tank). Tanks were scrubbed clean and fresh water (either control or 50µg/L U) was reintroduced within 20 mins of drainage to not stop the circulation of water in the fish tank for too long.

The pH was adjusted to 6.7 by adding HCl (4 M) in the synthetic EPA-water before use. The pH was not adjusted after the new water was transferred to the fish tanks, but daily measurements detected any change from the nominal value. Oxygen-saturation was kept close to 100 %. An O_2-pump was installed to keep this concentration of oxygen stable. The concentration of LMM U was maintained during the exposure by spiking the water with a U stock ($UO_2(NO_3)^2$ x 6 H_2O) (Sigma Aldrich). The U concentration had 40 % renewal every day (20µg/L), i.e. daily U-spikes.

2.3 Sampling

2.3.1 Water-samples

Water samples were collected throughout the experiment, to determine day-to-day variation in U concentration and fractionation. From early on, a rapid loss of bioavailable U in the exposure water was determined.

In addition to pre-exposed U measurements, the concentration of U was quantified almost daily throughout the experiment. The filtration methods used for water samples were 0.45µm-filtration, ultrafiltration and chelex. The total concentration of U in the tank was quantified through analysis of the unfiltered samples. The <0.45 µm-filtered samples did not include particles and thus separated the uranium bound in particles from the water. U colloids and LMM U species were then present in the filtered fraction. Unfiltered and filtered samples were collected every time the water was changed in the tanks. The unfiltered samples were collected with a pipette, and the filtered samples with a 0.45 µm-filter and a 10 mL syringe.

Uranium fractionization using ultrafiltration and ion chromatography were in addition used at day 2, 17 and 28 to separate colloids, low molecular mass species and low molecular mass-ions. Unfiltered, filtered and samples for the <10kDa and <10kDa chelex were collected and paired samples were completed. The pre-treatments for the <10kDa and <10kDa chelex were performed straight after water sampling. An ultrafiltration (UF) was completed of the <10kDa-samples. The unfiltered water samples were then run through a tube filled with hollow fibres. The ions and low molecular mass uranium (LMM U) weigh less than 10kDa and were then able to move through the ultrafilter in the hollow fibre and be collected in the filtrate. The LMM U species consisted of ions and low molecular mass complexes.

To determine the content of reactive ions of the ultrafiltration, paired water samples were run on the chelex. The chelex was a tube filled with a resin (chelex 100 resin, Bio-Rad). The resin contained particles, which had sodium-ions bound to the surface. The sample was led through the tube, and the more electron-negative ions than sodium switched position with the Na^+-ion. All positive charged U species in the sample was assumed to accumulate in the resin. A larger quantity of sodium in the <10kDa-chelex than in the <10kDa confirmed ion levels in the sample. If uranium was quantified in the sample after the chelex-treatment, complexes of uranium were present.

The samples were stored in 15mL-tubes. The water samples were stored in a temperature of +4^{o}C, to decrease the possibility of evaporation and fouling.

Calculations of the different U fractions:

Unfiltered sample = Total uranium

Unfiltered – 0.45 µm filtered = particulate uranium

0.45 µm filtered – <10kDa = colloidal uranium

<10kDa = LMM U species

<10kDa – <10kDa chelex = U cations

2.3.2 Fish samples

To determine the uptake and distribution of U in juvenile salmon, fish from each duplicate exposure were dissected on day 0, 2, 4, 8, 17 and 28. At sample point, fish (n=3) were transferred to tank (1L) containing Finquel anesthesia (MS-222 (Tricaine mesylate), 100mg/L, Scan Aqua AS), before measuring weight and length (**figure 2.2**). The length was measured from nose-tip to the end of fishtails. A picture was taken of each fish when the length was measured.

Blood samples were collected to determine glucose level (mmol/L). Changes of glucose-level in the blood indicated that the fish suffered from stress. The skin, gills, liver, kidney, stomach (w/content), brain, muscle and bone were dissected following the EMERGE protocol (Rosseland et al., 2001). When sampling the fish from the aqueous exposure, it was important to not contaminate the organs by contact with the skin. The equipment (metal tweezers of two different size, scalpel and a scissor) was cleaned in between tissues by using a paper towel. Aluminium foil was used to maintain a clean workspace, this foil was changed in between different exposures. The equipment was cleaned more thorough between exposure groups

with EtOH (70 % vv) to avoid cross-contamination. The samples were placed in pre-labelled 5 mL tubes and stored at -20^{0}C.

Figure 2.2: The left picture, length was quantified of fish. Right picture shows the fish before the dissection of internal organs (Pictures: B. Terum, 11.06.19).

2.4 Tissue and water analyses

2.4.1 Pre-treatments: water

All the water samples were sorted by date and given a unique number. Every volume was adjusted to 13 mL by using a pipette. The unfiltered and <0.45 µm-filtered samples did not undergo any pre-treatments in the lab.

Ultrapure HNO_3-acid was added to each sample to reach 10 % concentration (1.1 mL). The reference material was 1640a. Ten blank tests were pre-made. A house standard was used in the analysis (1643H). The water samples were further run on ICP-MS, together with the standards and the CRM.

2.4.2 Pre-treatments: fish

The frozen samples where freeze-dried overnight. The dried samples were weighed on a scale, by placing the tissue on the lid of the tube. The risk of contamination was then minimized. The sample was moved with either a plastic tweezer (organ) or a plastic pipette (only stomach). The equipment was rinsed with EtOH (70 %) between each organ. New equipment was used for every sample-point. The samples were weighted in increasing concentrations, to minimize the risk of contamination from the equipment.

2.4.2.1 Digestion

100 µL internal standard and 500 µL of ultrapure HNO_3-acid was added to each sample prior to the digestion. The samples were further placed in an oven at 90ºC for an hour to digest the tissues. After the digestion, the samples were diluted with 4.5 mL distilled water, i.e. filtered reverse osmosis (RO)-water. The final volume was 5 mL. The pre-treatments were then completed.

2.5 Data analysis

2.5.1 ICP-MS

The analysis was run on an Agilent ICP-MS QQQ – 8900 (mass spectrometry). The ICP-MS used ≈2 % HNO_3 as rinse solution between the samples and a 5 % HNO_3-solution as a liquid carrier. Either O_2-gas or He-gas was used as carrier-gas.

The results from the measurements on the ICP-MS were corrected based on analysis of the internal standard, online standard and quantifications of one known solution (drift). The internal standard detected loss of analyte (U) in the pre-treatments. The online standard was analysed regularly throughout the analysis, to correct for effects of matrix and drift in the instrument. The drift is a change in the instrument's measurement during analysis series, which cause a lower determined concentration in the sample.

The certified reference materials (ERM BB-422, IAEA 414 and 1640a) were used to analyse the accuracy of the measurements. A house standard (1643H) was analysed together with the water samples. See appendix 2 for further details about the analysis. Data explaining the concentrations of total organic carbon (TOC), chlorine (Cl) and nitrate (NO_3^-) during the exposure were not ready when this thesis was delivered. The weights were confirmed by plotting rubidium and total mass of the organs.

3.5.2 Formatting data and statistics

The results from the analysis on the ICP-MS were transferred to Excel spreadsheets. The drift was detected by analysing a known solution regularly throughout the analysis and calculated as a factor[2]. This factor was multiplied with the quantified concentrations in the unknown

[2] Calculation of drift is provided in Appendix 2 (eq. 3)

samples. The detection limit (LOD) and quantification limit (LOQ) were calculated. Precision of the method was quantified by calculating relative standard deviation (RSD) of the measurements.

The most optimal gas-carrier was chosen for each element. Then the results of the controls and 50 µg/L were isolated. The averages and standard deviations of both exposures were calculated for each day. The final results were plotted. To quantify the level of accuracy of the method, deviation of an CRM (certified reference material) and a house standard (1643H) were determined. Precision of the method was quantified by calculating relative standard deviation (RSD) of the measurements.

The exponential equations describing the uptake-rates in the different organs were calculated using SigmaPlot. The uptake-rate was assumed to follow a first order kinetic function with exponential rise to the maximum. The accumulation was assumed to have a rapid increase during the first days of exposure (approximately linear trend) and further flatten out, which indicated the concentration of uptake and elimination approached steady state. In this study, the uptake was assumed to reach steady state within 28 days of exposure.
A is the concentration after **x** days. $\mathbf{A_0}$ represents the steady state concentration. The **K**-value is the uptake coefficient. The uptake is affected by the already accumulated concentration of U in the tissue at the time. The uptake-rate is assumed to be large in the beginning of exposure and then slowly decrease over time until reaching the steady state concentration (Teien, pers comm)

$$\mathbf{2.}\ A = A_0\ (1- e^{-kx})$$

3. Results/discussion

3.1 Quality of the analysis on the ICP-MS

The concentrations of U in all the organs and water samples were above the quantification limits. The average LOQ[3] for organs was 0.00026 µg/g based on average 0.01g sample. LOQ was 0.0011 µg/l for water samples. If the concentration in the tissue was predicted to be low (example: muscle), a larger part of the organ was sampled (if possible) to lower the quantification limit on the ICP-MS. The total concentration of the element in the sample was then >LOQ.

3.1.1 Quality of the ICP-MS analysis (water)

The quantified concentrations and the certified concentrations of elements in the certified reference material (1640a) and the house standard (1643H) were listed in **table 3.1**. The results indicated good precision as the variations in the quantified concentrations of the different elements were low (RSD < 5 %), except for sulphur, in the house standard. The bias (%) were in the range of the nominal values (1643H) or <3 %, i.e. good accuracy. The determined concentrations of elements in certified reference material had a bias <3.2 %, also acceptable.

Table 3.1: Overview of the reference material used when measuring the water samples with certified concentrations and determined concentrations of house standard and CRM. Values presented in average ± std.dev. Calculations of RSD and bias in appendix 2 (eq. 7 and 4).

Ions	**1643H**				**1640a**			
	Nominal value	**Quantified value [4]**	**RSD%**	**Bias %[5]**	**Certified value**	**Quantified value [6]**	**RSD%**	**Bias %**
Na (mg/L)	20.7±0.26	19.9±0.3	1.4	-1.2	3.14±0.03	3.01±0.04	1.2	-3.2
Mg (mg/L)	8.0±0.10	8.08±0.09	1.1	Within	1.059±0.004	1.08±0.07	6.5	2.9
P (mg/L)	2.5	2.42±0.02	0.8	-2.4				
S (mg/L)	2.5	2.5±0.2	6.9	Within				
K (mg/L)	2.03±0.029	2.009±0.008	0.4	Within	0.580±0.002	0.558±0.001	1.1	-3.0
Ca (mg/L)	32±1.1	31.2±0.1	0.5	Within	5.62±0.02	5.4±0.3	6.2	-3.1
U (µg/L)	1	1.02±0.03	2.7	Within	25.35±0.27	25.0±0.6	2.2	-0.8

[3] Calculations of LOD and LOQ in Appendix 2 (eq. 8 and 9)
[4] N=4 for 1643H
[5] "Within" means within the range of house standard.
[6] N=4 for 1640a

3.1.2 Quality of the ICP-MS analysis (fish)

Two CRMs were used to determine the accuracy of the U concentration in fish samples. The bias was <5 % for all of the certified elements analysed.

Table 3.2: Certified concentrations and determined concentrations of the reference material, in addition deviation (bias, %) in certified reference material used when analysing fish samples. Values presented in average ± std.dev. Calculations of RSD and bias in appendix 2 (eq. 7 and 4).

Ions	ERM BB-422 (g/kg)	ERM BB-422-mea.[7]	RSD (%)	Bias (%)	IAEA 414	IAEA 414-mea.[8]	RSD (%)	Bias (%)
Na (mg/L)	2.80	2.81± 0.03	1.1	0.36				
K (mg/L)	21.4	20.5± 0.6	3.0	- 4.21				
Ca (mg/L)	0.342	0.33± 0.02	5.2	-2.94				
U-238 (ng/g)					86 - 92.5	77.5 ± 6.1	7.9	- 2.8

3.1.3 Discussion of quality of the ICP-MS (water and fish)

All the CRMs, both for water and fish, showed a bias (%) <5 %, which was an acceptable level of accuracy. The variation in quantified concentrations were low <5 % (RSD). The low values of RSD indicated good precision in the analysis of water and fish. Determination of uranium measurement (IAEA-414) showed RSD at 7.9 %, which indicated a larger variation in IAEA-414 standard than in the other CRMs. The dry-matter content in the IAEA-414 was 94.4 %. It would be preferred to use at least two CRMs for the determination of uranium to confirm the quantified U concentrations, especially if the CRM had a larger RSD than 5 %. In this experiment, it was important with a correct quantification of the U concentration in the samples. By comparing two CRMs, which both were certified for U, the credibility of the overall analysis would likely increase.

3.2 Characteristics of aqueous exposure

This chapter presents the water characteristics of the exposure water and the stability of the water chemistry throughout the experiment.

[7] N=5 for ERM bb-422
[8] N=6 for IAEA-414

3.2.1 General water quality

Moderate hard EPA-water was used in this experiment. The average dissolved ion concentrations in the control waters and in the 50 µg U/L waters (excluding particles (<0.45 µm) were listed in **table 3.3**. The RSD-values were <1 % for all elements.

The temperature, pH, conductivity, ammonium and carbon dioxide were not significantly different between the controls and 50µg U exposure waters. The temperatures were 14.6 ± 0.2 ^{o}C in both exposure experiments. The pH was 6.8± 0.1. The conductivity was at 380 ± 17 µS/cm for the control treatments and 370 ± 12 µS/cm for the 50µg U treatments. The relative fraction (%) of dissolved oxygen (DO) was significantly different between the two groups, but the fractions were close to 100 % in both treatments.

Table 3. 3 The average temperature, pH, conductivity, DO-, ammonium, CO_2-levels and ion composition in the exposure-waters. Concentrations were quantified using <0.45 µm-filtered water samples. Values are presented in average ± std.dev. [9] [10]

		EPA-moderate hard	**Control (ions: N=36)**		**50 µg U/L (ions: N=48)**	
	N= number of replicates	Nominal values	Quantified values	RSD (%)	Quantified values	RSD (%)
pH	N=17	6.7	6.8 ± 0.1	1.9	6.8 ± 0.1	1.8
Temperature (^{0}C)	N=17	<15	14.6 ± 0.2	1.3	14.6 ± 0.1	0.85
Conductivity (µS/cm)	N=17		380 ± 17	4.6	370 ± 12	3.2
Dissolved Oxygen (%)	N=17	≈100 %	97.6 ± 0.5	0.55	99.6 ± 0.2	0.15
NH4+ (mg/L)	N=10	≈98 %	0.7 ± 0.4	54	0.8 ± 0.5	57
CO2 (mg/L)	N=8		1.8 ± 0.5	27	1.8 ± 0.5	27
Cations (mg/L)	Ca	13.97	14.2 ± 1.4	10.2	13.4 ± 1.1	8.1
	K	2.10	2.63 ± 0.15	5.7	2.63 ± 0.13	5.0
	Mg	12.12	14.0 ± 0.70	5.0	13.84 ± 0.58	4.2
	Na	26.27	31.4 ± 1.6	5.1	31.2 ± 1.2	3.8
Anions (mg/L)	NH_3 (nitrate)	≈ 2 %	xx			
	Cl	37.4	xx			
	PO_4^{3-}	0	0.038 ± 0.020	54.3	0.038 ± 0.020	51.5
	SO_4^{2-}	56.87	89.3 ± 5.6	6.3	86.7 ± 4.0	4.6
	TOC	xx	xx			

[9] The concentrations of TOC, NO_3^- and Cl are not available at this time (22.01.20)
[10] Examples of calculations are provided in Appendix 2 (eq. 4, 5, 6 and 7)

The concentration of ammonium (NH_4^+) was 0.7 ± 0.4 mg/L in the controls and 0.8 ± 0.5mg/L in the U treatment waters. The exposures had the same CO_2- level at 1.8 ± 0.5 mg/L. The RSDs were <5 % for all parameters, except ammonium and carbon dioxide, which indicated stable water conditions in both exposures. The quantified concentrations of sulphate were higher in both treatments than the listed nominal concentration. The nominal concentration of phosphate was zero in EPA-moderate hard water, and the quantified concentrations in the treatments were low. The large relative standard deviations (RSD) indicated a large variation in the low concentration waters. A small variation to an already low concentration caused a larger RSD than if the average concentrations were higher.

3.2.2 Uranium concentration in total samples and fractions during experiment

The concentration of total uranium was on average 55± 22 µg U/L (n=32) in nominal 50µg U/L experiments and 0.027 ± 0.038 µg U/L (n=33) in the control. All total unfiltered samples analysed were included in these calculations. The total U concentration was fractionated to provide a better overview of the distribution of U species in the waters (**table 3.4**). Results showed that the concentrations of particulate, colloidal and low molecular mass (LMM) U species were 38 %, 37 % and 19 % respectively. The fish in the nominal 50 µg/l-treatment were on average exposed to 25 µg U particles, 29 µg U colloids and 17 µg LMM U species per litre. The average concentration of U cations was 17 ± 5 % of LMM U concentration, which indicate that the majority of the LMM U species were bound in complexes and did not exist as ions. The quantification of LMM cations indicated that only 1.13 ± 1.74 µg U/L of the total U concentration was expected to be potentially bioavailable.

Table 3.4: Concentrations of the paired fractions of uranium through the exposure. The percentage of fraction of the total concentration are listed in the parenthesis. LOD = 0.0003.[11][12]

Exposure	**Total U concentration**	**Particulate U**	**Colloidal U**	**LMM U**	**LMM U cations**
Control (µg U/L)	0.057 ± 0.074 (n= 6)	<LOD (n=5)	0.12 ± 0.19 (n=5)	0.06 ± 0.013 (n=6)	0.015 ± 0.022 (n=5)
50 µg U/L (µg U/L)	64 ± 34 (100 %) (n=7)	25 ± 12 (38 % of total) (n=6)	29 ± 20 (37 % of total) (n=6)	17 ± 16 (19 % of total) (n=7)	1.13 ± 1.74 (17 % of LMM) (n=6)

[11] The concentrations in **table 3.4** are a result of paired samples. The listed concentrations represent the average of the different conditions throughout the experiment, like fresh water (straight after change of water), two days after spiking (no change of water) and three days after change of water (daily spiking).

[12] The total concentrations should be the sum of particulate, colloidal and LMM-U, but are in both control and 50 µg U/L lower than the sum probably due to high standard deviations.

3.2.3 Stability of U concentration during the experiment

The U concentration varied somewhat throughout the experiment. The particulate fraction of uranium was significant and increased with time, probably due to sorption or complexation of U species to food particles. The LMM U species seemed to form colloids and being associated with particles, as the particulate and colloidal fraction increased while the LMM U fraction decreased. The initial analysis concentrations of LMM U species (day 0) decreased on day 2. To compensate for the decrease in the LMM U fraction and to ensure a more stable U exposure, the water was changed regularly and a daily spike of LMM U ions to the U treatment waters was performed in between the changes of water.

Before the U spiking was performed on a daily basis, the particulate U became the predominant fraction of U in solution (**figure 3.1**: day 2). After the daily spiking started, the concentration of particulate U did not reach the same level as at day 2, even if the water had not been changed for three days (**figure 3.1**: day 17 and 28). After the water was changed, the concentration of LMM U species was close to the nominal level, then the concentration of LMM U species decreased significantly likely due to rapid sorption of LMM-U species to particulate and colloidal matter. The concentration of LMM U species continued to decrease until the next U spike was added. This situation was repeated several times during the experimental period and the average distribution of the different size fractions were calculated based upon the average of the three different situations **(table 3.4).** Thus, U was mainly present as LMM species only short time after the exposure was initiated (**figure 3.1**).

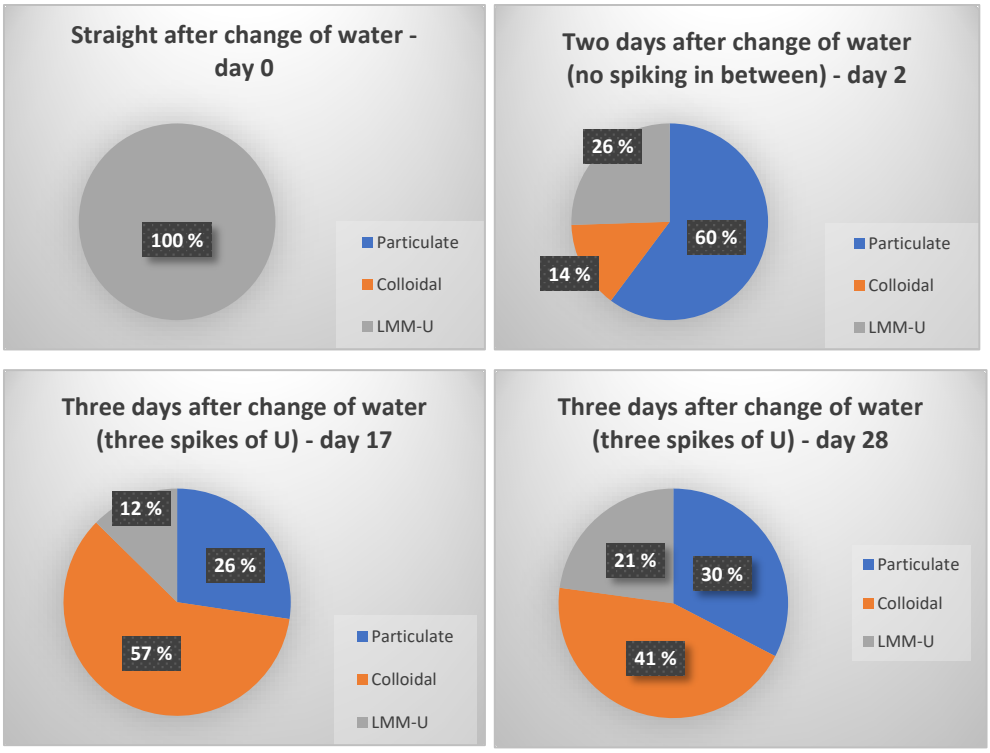

Figure 3.1: Pie-diagram of the fractional- distribution at day 2, 17 and 28. 1 - particulate U (blue), 2 - Colloidal U (orange), 3 – LMM-U (grey). Particulate U (n=4), colloidal U (n=2), LMM-U (n=2).

The water was changed several times between the days of sampling, and several U spikes were added. Thus, the U specie distribution changed dynamically during the experiment. An overview of the concentrations from day-to-day was then provided in **figure 3.2.** The "*<0.45 µm-filtered samples*"-graph imaged the U spikes with rapid increases in concentrations. The "*total concentration*"-graph indicated rapid binding of LMM U to particles after U spiking and water changes.

The "*Average U concentration per day*" (**figure 3.2**) imaged the quantified concentrations of U in the treatments throughout the experiment. An approximated linear trend described the total U concentration linked to the time of experiment (R^2=0.91), which showed an increased concentration by time. However, as demonstrated, a large fraction of total U was associated to particles, which was not expected to be bioavailable. The concentration of dissolved U (<0.45µm) had a much slower increase during the 28 days of exposure than the concentration of total U. The approximated linear trend describing the concentrations of dissolved U (<0.45µm) linked to time (day), was not significant (R^2 = 0.39). The exposure of the bioavailable U fractions was, however, more stable throughout the experiment, than the particulate concentrations.

The concentration of colloidal U and LMM U species (<0.45 µm) showed large variations during the first four days (seen as clear shifts in the graph). After the spiking started, the concentration became more stable and generally between 30 and 65 µg U/L. The uranium-spikes during the exposure could explain some of the variations observed in the fractions.

From the graph (**figure 3.2**), a change of water lead to a decrease of particulate uranium. After the change of water, the concentration of colloidal U and LMM U rapidly decreased due to rapid association of LMM U species to the excess DOM. The final unfiltered concentrations, at day 28, were around 100 µg U/L. The LMM U and colloidal U concentration were at the same time close to 70 µg U/L.

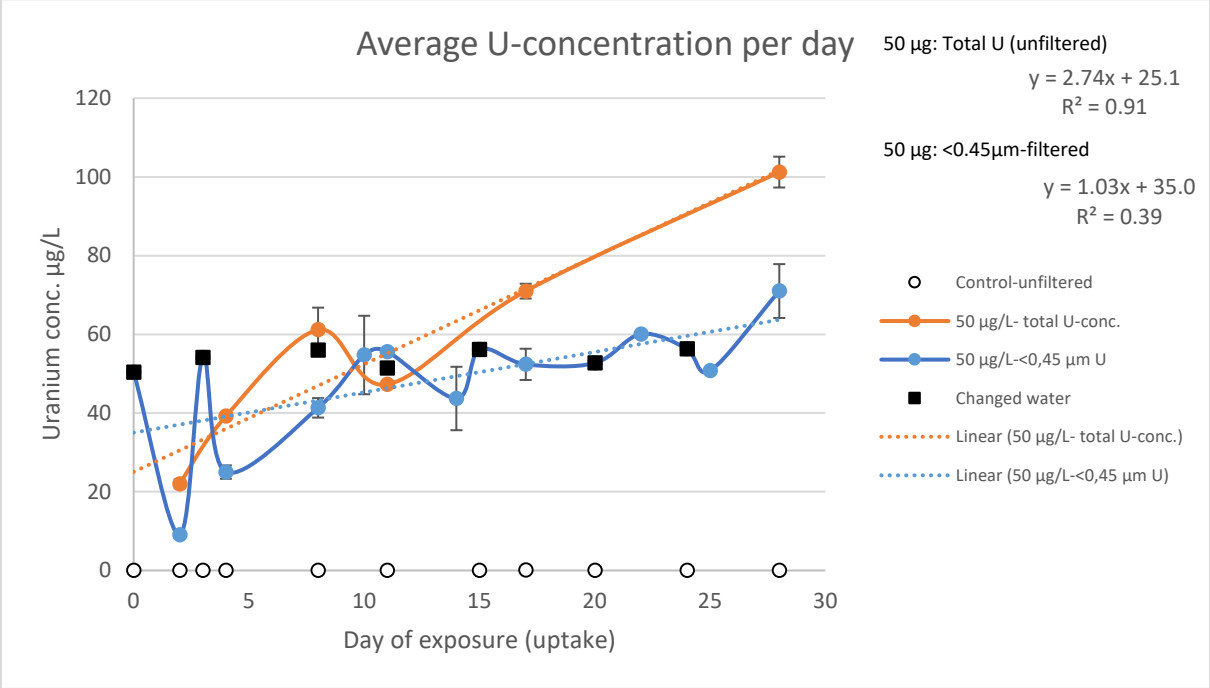

Figure 3.2: The average concentrations of uranium per day, in both treatments, were graphed. It was an even amount of time-points during the exposure. The "change of water"-days were marked as black squares. Error-bars with the individual standard deviation for each point were plotted.[13] Each time-point was based on the average ± standard deviation each day.

3.2.4 Discussion of aqueous exposure

The abiotic parameters (pH, temp, CO_2, NH_4^+, conductivity, anions and cations) were not significantly different between the two exposures, control and 50 µg U/L. It was a similar concentration (%) of dissolved oxygen in both treatments. The abiotic factors were not

[13] The graphs have a linear approximated trend line.

assumed to cause the fish additional stress, due to stable values (RSD < 10 %, except phosphate).

The pH in the water was constant during the exposure, which should have kept the ratio between the different uranium species relatively stable. The fractionation of the water samples showed variations in the concentrations of each U-fraction, thus the dynamic change throughout the experiment could be quantified. The U spikes consisted of 100 % LMM U. The uranyl ions did probably interact with the DOM from the feed and sorb to the surface of particles and colloids. When the water was changed, the particulate and colloidal U content was removed and the LMM U fraction had a 100 % concentration in the renewed water. The variations in U fractionation may then be explained by the change of water, daily U spiking and sorption to DOM. The data of total organic carbon in the water was unfortunately not available at present (February 2020).

The concentration of potentially bioavailable LMM U cations was only 17 % of the 19 % LMM-fraction quantified in the water samples. The U cation-concentration was 1.12 ± 1.74 µg U/L of total U concentration. The concentration of LMM species was low (17 ± 16 µg U/L), compared to the nominal U concentration of 50 µg U/L. The total U concentration was higher than nominal U concentration throughout the exposure, but total U concentration was not assumed to affect the uptake of U in fish (Franklin et al., 2000). The large variations (standard deviations) were linked to the dynamic change of U species throughout the exposure. Fractionation of U were only conducted at three timepoints (day 2, 17 and 28) during the experiment. When considering the dynamic change of U species would it be preferable if more water samples were fractionated.

3.3 Characteristics of uranium uptake in salmon (*Salmo salar)*

The following sections include the analysis of the fish tissues, i.e. the quantification of U concentration in the tissues analysed (gill, stomach w/content), liver, kidney, skin and muscle), calculation of uptake-rate and bioconcentration factor and a comparison between tissues.

3.3.1 Growth and glucose level

The fish did not experience significant growth during the 28 days of exposure (overlapping standard deviations), neither in the control nor the U exposure. The fish weights in both of the treatments had the same variation (RSD (%) ≈ 30). The lengths of the fish had smaller variation, a factor of 9 % (RSD, %), in both treatments.

The quantified concentrations of glucose in the blood samples of each fish varied more between the fish (n=6) in the same group, than in between the three different time-points. The mean of each group was 3.7 ± 1.6 mmol/l (control) and 3.3 ± 1.6 mmol/l (U treatment). This did not change significantly between the different time-points. The concentrations were equivalent to listed normal values of fish (3-6 mmol/l) (Kroglund et al., 2001) and indicated low levels of stress in the fish.

3.3.2 Uranium in different tissue

The U concentration in the control fish was low. The highest concentrations of U in tissues from the control-group were 0.011 ± 0.004 µg U/g tissue (stomach w/content) and 0.0084 ± 0.0054 µg U/g tissue (gill). The accumulation of uranium was significant in all tissues after the 28 days of U exposure (**Table 3.5**). The highest U concentration was quantified in the gills (5.9 ± 0.9 µg U/g tissue) after 28 days of exposure. The liver had the lowest concentration of the analysed organs after 28 days.

Table 3.5: Average concentrations in different tissues after 28 days of exposure.

Treatment	**Gill (n=6)**	**Skin (n=5)**	**Liver (n=5)**	**Kidney (n=6)**	**Stomach w/content (n=5)**	**Muscle (n=5)**[14]
Control (µg/g dw)	0.0084 ± 0.0054	0.0063 ± 0.0058	0.0009 ± 0.0003	0.0048 ± 0.0012	0.011 ± 0.004	0.0017 ± 0.0008
50 µg U/L (µg U/g dw)	5.85 ± 0.884	0.598 ± 0.151	0.023 ± 0.006	0.228 ± 0.157	0.829 ± 0.761	0.135 ± 0.089

3.3.2.1 Gill

The accumulation of U was already significant in the gills at day two of exposure, and it increased over time during the 28 days. It was further a significant increase from day 2 to day 17, however not a significantly difference in U concentration between day 17 and day 28. The concentrations were about 5.9 µg U/g gill tissue (dw) after 28 days of exposure. The

[14] Concentrations are above the average LOQ for organs at 0.00026 µg/g.

variations between the fish at each sample point were small (RSD (%): 10-26). It was not a significantly U accumulation in the control treatment.

SigmaPlot estimated steady state in gills to be 30.3 µg U/g. The exponential rise to the maximum equation had a P=0.46 and a R^2= 0.91. The model was not significant, and more data points were needed to identify steady state and the uptake coefficient (K-value). The uptake-rate was estimated to be 0.24 ug U/g tissue/day[15]. The linear trend (R^2=0.95) plotted in the graph (**figure 3.3**) had a slope of 0.22 µg U/g tissue/day, which was approximately the same uptake-rate as the estimation from SigmaPlot. The uptake-rate in the gills was estimated to be between 0.22 and 0.24 µg U/g tissue/day. According to the estimated steady-state concentration of U in the gills, the quantified concentration at day 28 was still far from reaching steady state in the organ, BCF was then not calculated.

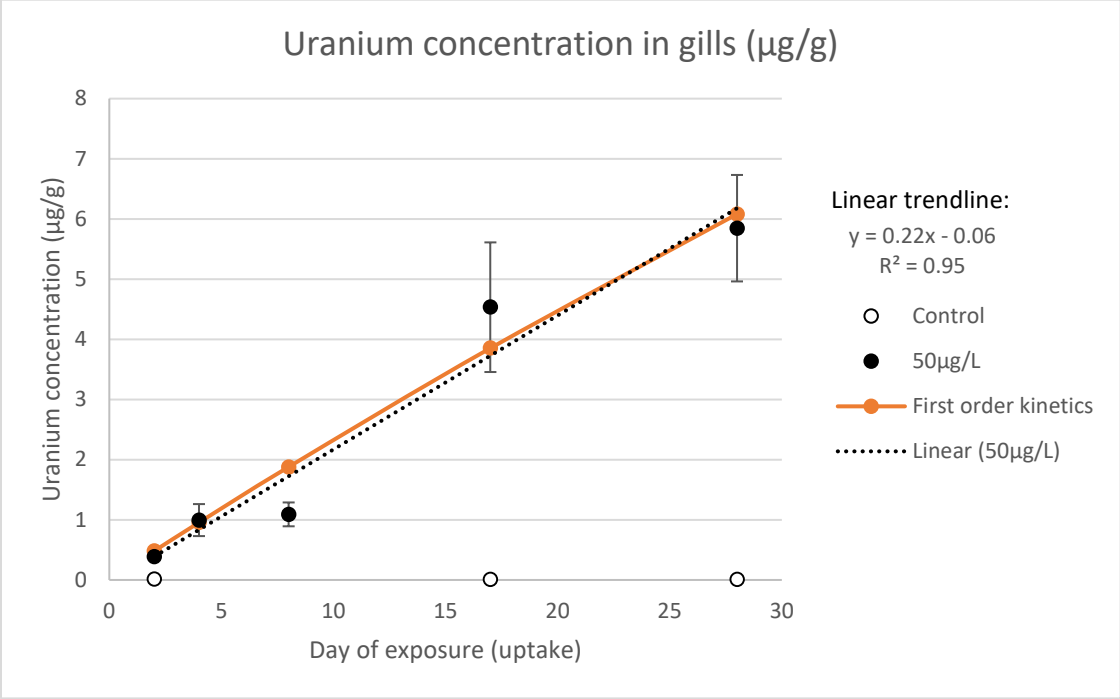

Figure 3. 3 Overview of the accumulation of U in gills during the exposure. The fish were exposed to an average of 50.1 ± 21.0 µg/L U (based upon <0.45 µm-filtered water samples (n=48) [16]. The graph "First order kinetics" image exponential rise to a maximum, predicted by SigmaPlot.

3.3.2.2 Liver

The concentration of U in the exposure was significantly higher than in the control after 4 days of exposure. It was a significant increase in U concentration between day 8 and day 28.

[15] Calculations in Appendix 3
[16] All concentrations are given in µg U/g tissue (dw)

The large variation between the fish at day 17 made it difficult to identify if the increase was significant before 28 days of exposure. The variation within the concentrations from the same day differed from small to large (RSD (%): 28-80). It was not a significant accumulation of U in the control treatment.

The concentration of uranium in the body increased during the exposure, which increased the elimination process in the liver. An increase of U concentration in the liver was then likely to be seen during the uptake period. The increased concentration of U in the liver supported the theory in which the liver is an essential organ in the detoxification mechanism of uranium (Cooley and Klaverkamp, 2000), but the accumulation was in comparison to the other organs still low at day 28.

The U accumulation was assumed to follow an exponential rise to the maximum and was estimated to reach a concentration of 0.029 µg U/g liver at steady state (SigmaPlot). This estimation had a low P-value (<0.05) and a R^2=0.60. The uptake-rate was estimated to be 0.0016 µg U/g per day. The linear trend (R^2=0.98) plotted in the graph (**figure 3.4**) had a slope of 0.0007 µg U/g tissue/day, which indicated a slower uptake-rate compared to the estimation from SigmaPlot. The uptake was higher than the prediction from the linear trend, as the accumulation started to reach steady state. The uptake-rate in the liver was assumed to be closer to 0.0016 µg U/g tissue/day. The apparent BCF was quantified for liver at day 28, because the concentration in the tissue was close to the estimated steady-state concentration. The apparent BCF in liver was 0.52 L/mg (based upon the 0.45µm-filtered U conc.) and 6.3 L/mg (based upon the U cations conc.).

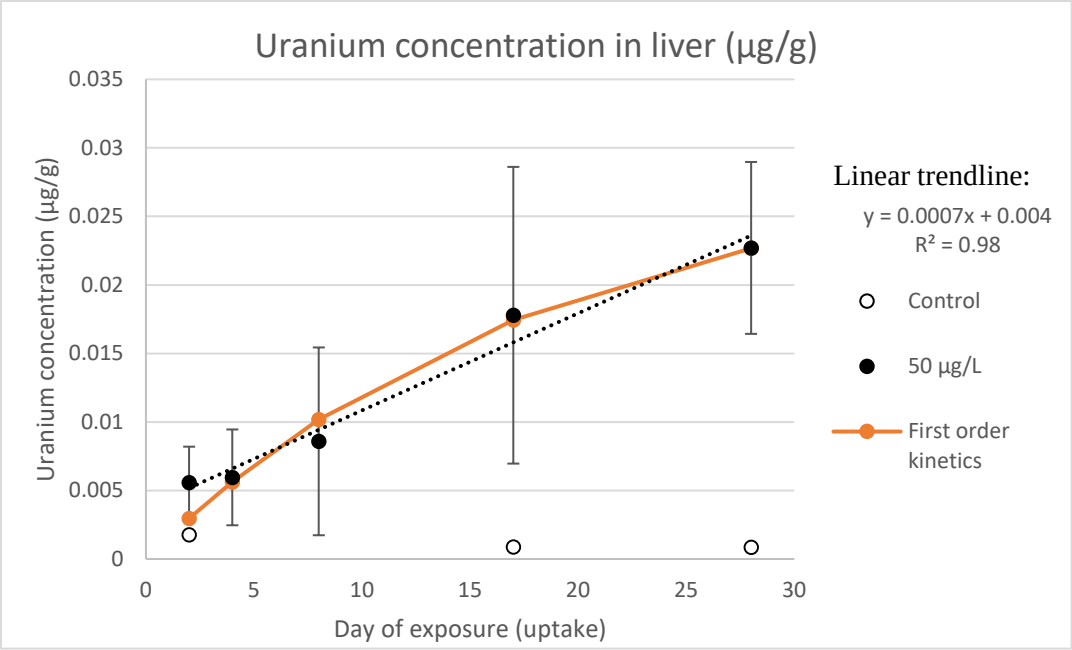

Figure 3. 4 Overview of the U accumulation in liver during the exposure. The fish were exposed to an average of 50.1 ± 21.0 µg/L U (based upon <0.45 µm-filtered water samples (n=48). [17] The graph "First order kinetics" image exponential rise to a maximum, predicted by SigmaPlot.

3.3.2.3 Kidney

It was a significant accumulation of U in the kidney during the 28 days, but the variations between the parallel fish at each day were large. The concentrations were significantly different between day 2 and day 17. The concentrations at each sample point had large variations (RSD (%): 25-140), this may have indicated some kind of contamination. Day 4 had the largest variation between concentrations (RSD=137 %). Compared to day 4, the variations between the parallel fish from day 8 and 17 were much lower. It was not a significantly accumulation of U in the control.

The kidney filtrates the blood, which may contain U if the element is taken up in the body (Goulet et al., 2012). An increase of U concentration in the kidney was then likely to be seen during the uptake period. U concentrations in the kidney may increase further in the depuration period, a similar trend as in the liver was predicted.

The accumulation was assumed to follow an exponential rise to the maximum. However, the estimation was far from being validated with a P-value close to 1 and R^2=0.41. The accumulation was then far from reaching steady state at day 28, and BCF was not calculated.

[17] All concentrations are given in µg U/g tissue (dw)

The linear trend (R^2=0.94) plotted in the graph (**figure 3.5**) had a slope of 0.0054 µg U/g tissue/day, which indicate the uptake-rate in kidney.

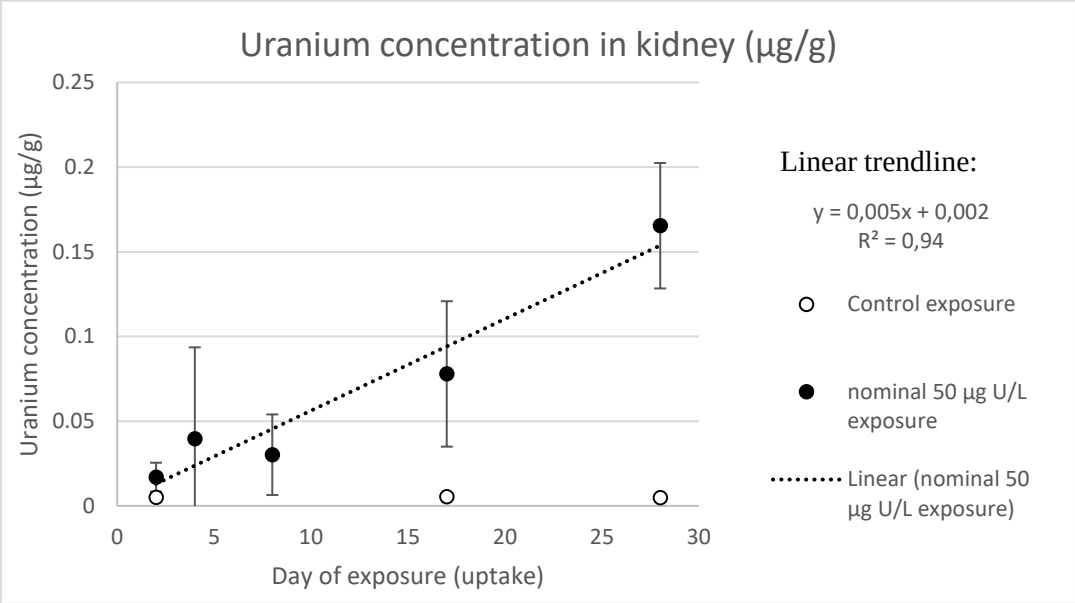

Figure 3.5: Overview of the U accumulation in kidney during the exposure. The fish were exposed to an average of 50.1 ± 21.0 µg/L U (based upon <0.45 µm-filtered water samples (n=48). [18]

3.3.2.4 Skin

It was a significant accumulation of U in the skin. It was significantly different concentration between day 2, day 17 and day 28 (not overlapping standard deviations with control). The range of variations between the parallel fish at each timepoint were large (RSD (%): 25-90). The quantified concentration at day 2 had a large variation (RSD (%): 90). It was not a significantly accumulation of U in the control.

The high values of RSD indicated large variations within the parallels. At day 2, two of the fish had concentrations at 0.118 and 0.204 µg/g compared to 0.021-0.055 µg/g in the other fish. Cross-contamination during sampling due to handling could be possible, as it was difficult to not touch the skin before sampling. Though, at this time point, did only the gill and stomach (w/content) have higher concentrations than the skin, and contamination from these tissues were unlikely. Mucus from the surface of the skin may have been removed when handling the fish before sampling, which made the quantified concentration of U lower than original. The water had quite high concentrations of particulate U at day 2, but the particulate U compounds were assumed to be less bioavailable and were not likely to sorb to the surface

[18] All concentrations are given in µg U/g tissue (dw)

of the skin. The U concentration at day 28 was about twice as high as at day 17, similar to the increase between day 8 and day 17, which indicated a low effect of the increased particulate concentration of U in the tanks.

The accumulation of U was assumed to follow an exponential rise to the maximum. However, the estimation was far from being validated with a P-value close to 1 and R^2=0.81. The accumulation was then far from reaching steady state at day 28, and BCF was not calculated. The linear trend (R^2=0.97) plotted in the graph (**figure 3.6**) had a slope of 0.020 µg U/g tissue/day, which was the estimated uptake-rate in skin.

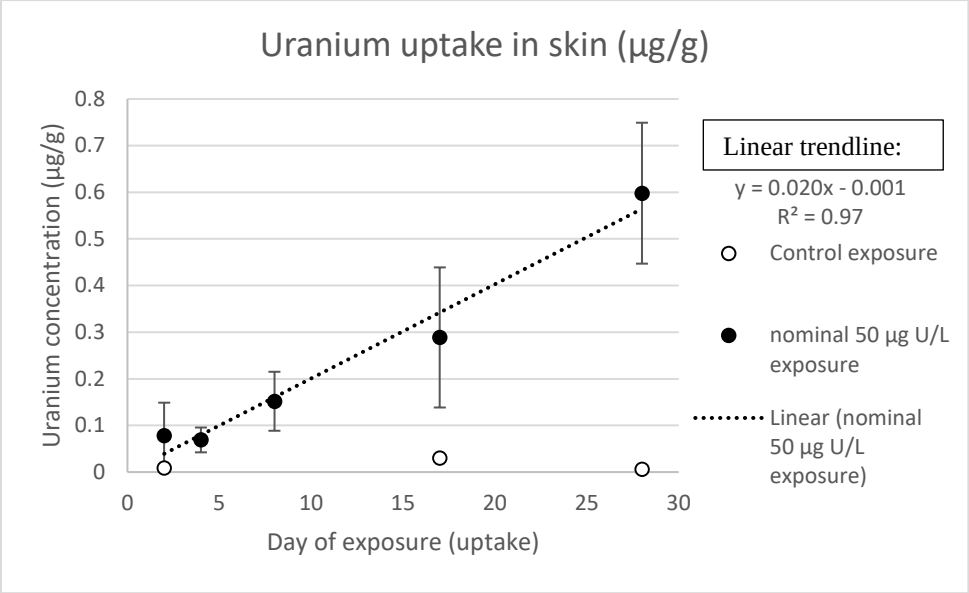

Figure 3.6: Overview of the U accumulation in skin during the exposure. The fish were exposed to an average of 50.1 ± 21.0 µg/L U (based upon <0.45 µm-filtered water samples (n=48). [19]

3.3.2.5 Stomach w/content

The plotted concentrations showed a significant accumulation of uranium in the stomach (w/content) between day 4 and day 8, compared to the control fish. The U concentrations in the stomach (w/content) had large variations between the parallel fish. The RSDs were between 47-92 %. It was not a significant increase in the U concentration in the organ after day 8. The concentrations from day 4, 8 and 28 were significantly different from the control. It was not a significantly accumulation of U in the control.

[19] All concentrations are given in µg U/g tissue (dw)

The largest variation (highest RSD) between the quantified U concentrations was at day 28. The concentrations then differed from 0.178 to 1.95 µg U/g stomach (w/content). Fish in freshwater do not drink water to maintain the ion-balance in the body (Rosseland, 2000). The particulate U concentration was high at day 2 (≈60 %) and lower (≈30 %) at day 17 and 28, which may explain the high concentrations of U in the stomachs (w/content). The stomach was not emptied pre-sampling, which may be seen as higher U concentrations in the stomach than what was actually accumulated in the tissue. On the other hand, the fish were not fed prior to sampling and the last food was given about 18 hours before sampling.

The U accumulation was assumed to follow an exponential rise to the maximum and was estimated to reach the concentration of 0.73 µg U/g stomach (w/content) at steady state (SigmaPlot), which was lower than the quantified concentration at day 28 (0.83 ± 0.76 µg U/g tissue). It seemed like steady state of U accumulation was reached in stomach. This estimation had a low P-value (<0.05) and a R^2=0.31. The uptake-rate was estimated to be 0.073 µg U/g tissue/day. The linear trend (R^2=0.98) plotted in the graph (**figure 3.7**) had a slope of 0.059 µg U/g tissue/day, which indicated a slower accumulation of U than the estimation from SigmaPlot. The graphs indicated that stomach (w/content) reached steady state faster than the linear trend estimated. The uptake-rate in the stomach (w/content) was estimated to be 0.059-0.073 µg U/g tissue/day. The bioconcentration factor was 18.5 L/mg and based upon the particulate U concentration at day 28.

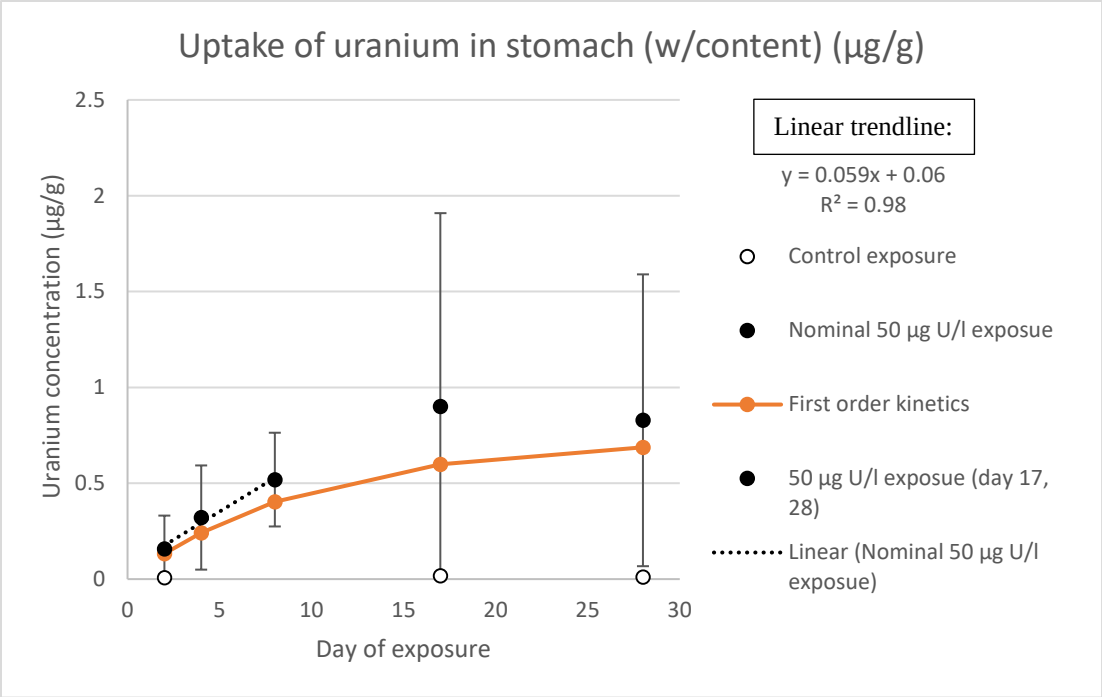

Figure 3.7: Overview of the U accumulation in stomach (w/content) during the exposure. The fish were exposed to an average of 24.9 ± 11.9 µg/L U (based upon the average particulate U concentration in the water (n=6). [20] The graph "First order kinetics" image exponential rise to a maximum, predicted by SigmaPlot.

3.3.2.6 Muscle

The accumulation of uranium in the muscle was significant during the 28 days of exposure. It was a significant accumulation of U at day 4 in the exposure period. The concentrations were low, and the variations were large between the parallel fish from each timepoint (RSD (%): 65-73). The RSD at day 17 was high (93 %), due to great variation in the concentrations (0.012- 0.096 µg U/g). The average U concentration at day 28 was 0.10 ± 0.05 µg U/g. It was not a significantly accumulation of U in the control.

The U accumulation was assumed to follow an exponential rise to the maximum. However, the estimation was far from being validated with a P-value close to 1 and R^2=0.64. The accumulation of U was far from reaching steady state after 8 days of exposure, and BCF was then not calculated. The linear trend (R^2=0.87) plotted in the graph (**figure 3.8**) had a slope of 0.0049 µg U/g tissue/day, which indicated the uptake-rate in muscle.

[20] All concentrations are given in µg U/g tissue (dw)

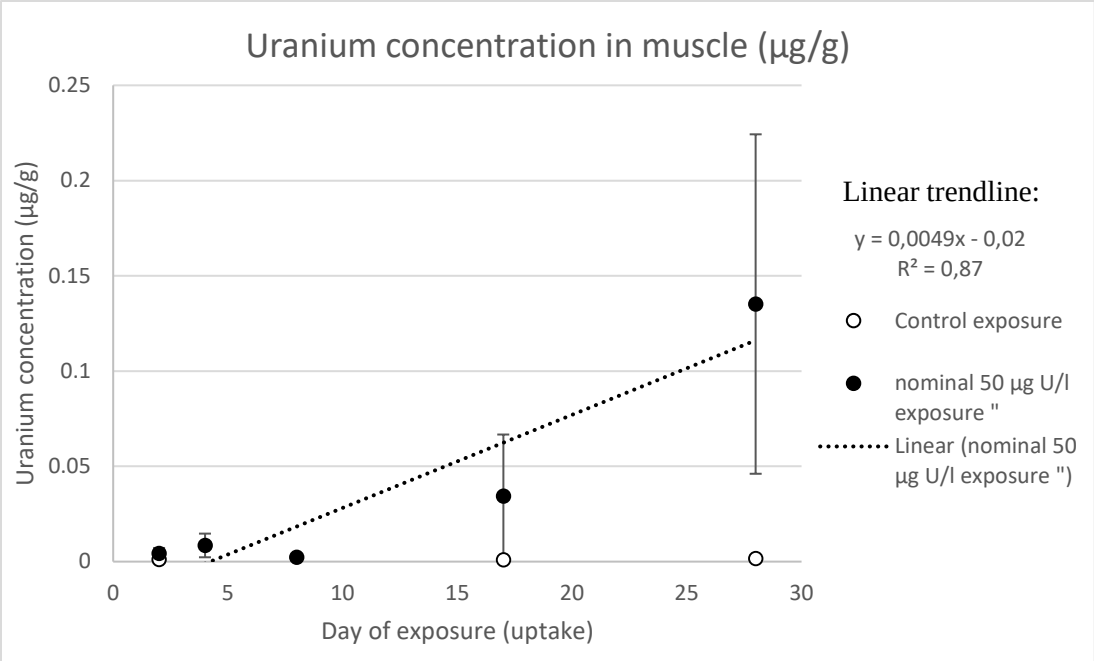

Figure 3.8: Overview of the U accumulation in muscle during the exposure. The fish were exposed to an average of 50.1 ± 21.0 µg/L U (based upon <0.45 µm-filtered water samples (n=48). [21]

3.3.3 U distribution in different organs

It was a significant accumulation of U in all tissues analysed. Uranium was taken up in the fish, which was determined by significant concentrations in the muscle, kidney and liver (internal organs). It was assumed that the concentration of U in an organ would increase until reaching a level of steady state. It was not a significant accumulation of uranium in any of the organs in the control treatments.

The bioconcentration factors were estimated for liver and stomach (w/content). All of the organs, except stomach (w/content) and liver, were far from reaching the estimated organ-specific, steady state-concentrations. Only stomach (w/content) reached the estimated level of steady state in accumulation/elimination of U. The estimated BCF of stomach (w/content) was 18.5 L/mg (based upon the particulate U conc.) at day 28. The estimation had large uncertainties because the content in the stomach was not quantified. It was, most likely, particulate U because the fish did probably eat the particles in the water and not drink significant amounts of water (Rosseland, 2000). The quantified U concentration in the stomach (w/content) at day 28 had large variation and overlapping standard deviations with day 17, it is then not known if the concentration in the stomach (w/content) passed the

[21] All concentrations are given in µg U/g tissue (dw)

estimated concentration of steady state or not. It was not determined whether or not the quantified U concentration actually accumulated in the tissue.

The estimated BCF of liver was 0.52 L/mg (based upon the 0.45µm-filtered U conc.) and 6.3 L/mg (based upon the U cations conc.). Both estimations were higher than the previous calculations of BCF in the field (Liver: 0.25-5.6 L/kg) (Salbu et al., 2013; Strømman et al., 2013; Lind et al., 2013). The other organs were estimated to be far from reaching their steady state in uptake of U, and BCF was then not calculated.

The largest concentrations of U were quantified in the gills (5.85 ± 0.884 µg U/g tissue). The concentration in the gills correlated (R^2=0.61-0.69) with the concentrations in the kidney, liver and muscle (**figure 3.9**). The U concentration in the gills was on average 37, 268 and 63 times higher than the U concentrations in kidney, liver and muscle at day 28. The U concentration in the gills correlated with U concentration in the skin (R^2=0.72), which was assumed because of the direct sorption of U from water. The U concentration in the gills were on average 13 times higher than in the skin at day 28.

The U concentration in the gills did not correlate with the U concentration in the stomach (w/content) (R^2= 0.098). The U concentration in the gills were on average 14 times higher than in the stomach (w/content) at day 28. The U concentrations in the stomach and the internal organs (kidney, liver and muscle) had weak correlation (R^2=0.15-0.22), which may have indicated a low uptake of U through the stomach even if the second highest concentrations of U were quantified in the stomach (w/content) (0.83 ± 0.76 µg U/g tissue). The U concentration in the stomach was on average 5, 36 and 8 times higher than the U concentrations in kidney, liver and muscle at day 28.

The U concentrations in the skin and the internal organs (kidney and muscle) correlated (R^2= 0.69-0.76). The U concentration in skin and liver had less correlation (R^2=0.49). The quantified concentrations in the skin was third highest (0.598 ± 0.151 µg U/g tissue). The U concentration in the skin was on average 4, 28 and 7 times higher than the U concentrations in kidney, liver and muscle at day 28. Uptake of U through the skin was then not excluded in aqueous exposure to Atlantic salmon.

The U concentrations in the liver and kidney correlated (R^2= 0.62), and at day 28 was the U concentration in the kidney on average 8 times higher than in the liver. The U concentration in the liver and the muscle correlated (R^2= 0.53), and at day 28 was the U concentration in the liver on average 0.3 times higher than in the liver. The kidney and muscle had a correlation (R^2= 0.60), and at day 28 was the U concentration in the liver on average 2 times higher than in the liver. The correlation between internal organs was assumed to be high, because the U taken up in the blood, was assumed to accumulate similarly in kidney and liver. The accumulation of U in muscle was assumed to be lower than kidney and liver, because of the elimination process.

The kidney, muscle and liver had the three lowest accumulated U concentrations, respectively, of the organs analysed. The concentrations were increasing in the internal organs, which were an estimated development due to accumulation and uptake of U in the fish. An elimination of U from the body (blood, muscle) was assumed to increase the concentrations in kidney and liver. The standard deviations in the kidney were high, which made the difference in accumulation compared to the liver less significant. The biodilution in the fish was not quantified, because the fish did not experience a significant growth during the 28 days.

The calculated uptake-rates from the estimated, organ-specific, steady state concentrations showed that the significantly highest uptake-rate of U accumulation was in the gills (0.22-0.24 µg U/g tissue/day). Stomach (w/content) and skin had second and third highest uptake-rates at 0.059-0.073 and 0.020 µg U/g tissue/day, respectively. The internal tissues had uptake-rates at 0.0054 µg U/g tissue/day (kidney), 0.0049 µg U/g tissue/day (muscle) and 0.0016 µg U/g tissue/day (liver).

The last U spike was probably unnecessary because of the already high concentration of LMM U in the tanks. This increase in concentration had probably small effect on the uptake through the gills and stomach, because the fish were sampled within a few hours. It may have affected the accumulation and concentration on the skin and gills, due to the direct contact with the water. A change of water before the last couple of days of exposure, would have lowered the particulate concentration of U at day 28.

Table 3.6: Correlation between possible uptake-routes (concentration) of U (gills, skin, stomach) and accumulation (concentration) in internal organs (kidney, liver and muscle). Concentrations were plotted in µg/g tissue. The U concentration were compared between two organs in the same fish. Correlations between U accumulation in internal organs (liver-kidney, liver-muscle, kidney-muscle) were quantified, as well as correlation between uptake-routes (gill-skin and gill-stomach).[22]

Organs	Correlation	R^2	Correlation factor of U concentrations in different tissues at day 28
Gill-kidney	Y = 0.022x + 0.009	0.62	[gill] = 37 x [kidney]
Gill-liver	Y = 0.003x + 0.004	0.62	[gill] = 268 x [liver]
Gill-muscle	Y = 0.016x - 0.011	0.69	[gill] = 63 x [muscle]
Gill-skin	Y = 0.081x + 0.027	0.72	[gill] = 13 x [skin]
Gill-stomach (w/content)	Y = 0.062x + 0.28	0.098	[gill] = 14 x [stomach w/content]
Stomach (w/content) -kidney	Y = 0.068x + 0.036	0.22	[stomach w/content] = 5 x [kidney]
Stomach (w/content) -liver	Y = 0.007x + 0.009	0.15	[stomach w/content] = 36 x [liver]
Stomach (w/content) - muscle	Y = 0.045x + 0.010	0.20	[stomach w/content] = 8 x [muscle]
Skin - kidney	Y = 0.241x + 0.008	0.69	[skin] = 4 x [kidney]
Skin - liver	Y = 0.030x + 0.005	0.49	[skin] = 27 x [liver]
Skin - muscle	Y = 0.175x – 0.011	0.76	[skin] = 8 x [muscle]
Kidney - liver	Y = 0.12x + 0.01	0.62	[kidney] = 8 x [liver]
Liver - muscle	Y = 3.40x - 0.012	0.53	[liver] = 0.3 x [muscle]
Kidney - muscle	Y = 0.542x - 0.006	0.60	[kidney] = 2 x [muscle]

3.4 Coherence of U concentration in the water and uptake in fish tissue

The concentrations of ions were the same in the control- and 50 µg U/L-treatments. The batch-water was the same in both exposures. The uranium was added after the salts, and only for the isolated U water. It was not expected any difference between the concentrations of ions in the two exposures. Small differences were quantified from the nominal values. The abiotic parameters of water quality were not significantly different. The constant water-conditions provided less stress for the fish during the experiment. Low glucose-concentrations indicated that the fish were not stressed. The fish in the control-exposure had lower concentrations of U in the tissues than in the water (0.057 ± 0.074 µg/L), but above the average quantification limit of organs (0.26 ng/g).

The total concentration of U, as well as the LMM-U concentration, increased during the 28 days of exposure. It was a dynamic change in fractionation of uranium throughout the

[22] Example on calculation of correlation factor in Appendix 4.

exposure, due to change of water, rapid binding to organic matter (mainly from feed) and daily U spikes. Regular exchanges of water were required to reduce the concentration of particles in the water, which further reduced the U sorption to the food particles. It was effective to perform regular U spikes to maintain a more even concentration of LMM U in the water solution.

The concentration of U cations was low, only 1.12 ± 1.74 µg/L, and only 17 % of the LMM U concentration in the U treatment. The fraction of uranyl-ions is relatively low at pH 6.8 ± 0.1 (Goulet et al., 2012). It was several procedures in the pre-treatments of the water samples and the analysing time on the ICP-MS, which made it difficult to perform a more regularly fractionation of the water. With only three time-points for U fractionation of the water, it was difficult to know the exact U concentration of each fraction at a specific time. More time-points with fractionation of water samples would be favourable to determine the dynamical change of U fractions in the water in a broader sense.

The high concentrations of particulate uranium in the water (38 %) changed the focus in the thesis to include the aspect of potential uptake of U through the stomach. High concentrations of particulate bound uranium were assumed to increase U accumulation in the stomach if the fish ate the particles. An increasing U concentration during exposure was identified in the stomach. The concentration of U in stomach (w/content) did however not correlate with other tissues, which also supports the theory that the uptake in stomach was different from uptake in gills. The fish seemed to have eaten U contaminated particles. The large variation in quantified U concentrations can be seen as individual differences in the consumption of U particles between fish. The reduction of particulate uranium through water exchanges did likely lower the potential uptake of U through the stomach.

The accumulated U concentrations increased in all tissues during the exposure to waterborne U. The accumulation of U was significant in all of the analysed organs (gills, stomach, skin, kidney, liver, muscle) after 4-8 days of exposure to 1.12 ± 1.74 µg/L of U cations. The skin and gills had significant accumulation of U at day 2. The accumulated concentration in the gills was significantly higher than in all other organs analysed. The organs in direct contact with the U contaminated water or particles (gills, stomach and skin) experienced the highest accumulations. The internal organs (muscle, kidney and liver) accumulated also U, which demonstrated that U was transported from water to internal tissues.

The uptake-rate differed between the organs. The estimated uptake-rates were in general lower for the organs with longer uptake-routes (muscle, kidney and liver) than for the organs with direct contact with the water and a shorter uptake-route (gills, skin and stomach w/content).

The ability to reach steady state of U accumulation in organs was likely affected by the unstable U exposure throughout the experiment. The lack of reached steady states in the accumulation resulted in few calculations of bioconcentration factors in the different tissues. Only liver and stomach were close or actually reached the estimated, organ-specific, concentration of steady state. The low concentration in the liver may be explained by the fact that few organs reached the estimated steady state concentration and the further elimination of U was then low. The other organs (gill, kidney, skin and muscle) had estimated uptake-rates similar to the slope of a linear trend, which indicated that the organs were in the start of uptake, and far from reaching steady state.

The stomach (w/content) had a BCF of 18.5 L/mg (based upon the particulate U concentration) at day 28. The apparent BCF in liver was 0.52 L/mg (based upon the <0.45µm-filtered U conc.) and 6.3 L/mg (based upon the U cations conc.). The factors were significantly higher than the listed values from other laboratory experiments (Goulet et al., 2012) and quantified values from field experiments (Salbu et al., 2013; Strømman et al., 2013; Lind et al., 2013). The BCFs from previous studies were calculated based on the total dissolved U concentration (<0.45µm-filtered) at a higher pH (8-8.5). The pH is known to affect the solubility and speciation of U in water (Goulet et al., 2012; Franklin et al., 2000; Giblin et al., 2015). The speciation affects the bioavailable fraction of U, which further affects the uptake of U in the fish. The uptake of U affects the steady state concentration, and then the BCF. The fraction of uranyl-ions in water at pH >8 is known to be small (Goulet et al., 2012). A smaller U concentration in the fish tissue at steady state is assumed to generate a lower BCF-value. The BCF is then assumed to vary between different pH, due to different levels of bioavailable species present in the water. The 0.45µm-filtered water includes all dissolved U species and not just the bioavailable fraction (U cations), which is why the U-cation concentration was used to calculate the BCF in the liver.

There were three possible uptake-routes of U in this study: through the gills, stomach and skin. It was assumed that the bioavailable U fraction (U cations) was taken up through the gills, and the accumulation of U was the highest in the gills even if the quantified U cation-concentration was low. The highest uptake-rate was determined in the gills (0.22-24 µg U/g per day). The correlation between the concentration in the gills and the concentrations in the internal organs (kidney, liver and muscle) were significant (R^2=61-69 %).

The stomach (w/content) had the second highest U uptake-rate (0.059-0.073 µg U/g per day) of the organs analysed, but the concentration at day 28 was more than 14 times lower than what was present in the gills. The stomach (w/content) was the only organ which reached the estimated concentration of steady state. The only assumed uptake of U from the stomach was from U contaminated particles, which had a high concentration (38 %) throughout the experiment. The correlation between the accumulated concentration in the stomach and concentrations in the internal tissues (kidney, liver and muscle) were, on the other hand, low (R^2=15-22 %). It is not known, whether or not, the uranium quantified in the stomach actually accumulated in the tissue or if it just passed the intestines slowly without being taken up in the blood. The content was not separated from the stomach and analysed separately, i.e. quantification of U concentration and fractionation, which leaves this question unanswered. However, the uptake through the stomach was assumed to be low (Bleise et al., 2003; WHO, 2001).

The skin had the third highest U accumulation after 28 days of exposure, but the concentration was about 13 times lower than in the gills at the same time point. The bioavailable fraction (U cations) was assumed to accumulate in the skin. It was also assumed that both the gills and the skin absorbed U directly from water, due to negative charged surfaces (Rosseland, 2001). The correlating U concentrations in the gill and skin supported the assumption (R^2=0.72). The U concentrations in the skin and the internal tissues (kidney and muscle) had strong correlation (R^2=69-76 %), and uptake of U through the skin was then not excluded in aqueous exposure to Atlantic salmon.

The main uptake of U was predicted to be through the gills, due to significantly higher accumulated concentration and higher uptake-rate than in the skin and stomach. Barillet (2007) assumed the main uptake-route of waterborne U to be through the gills, which was the

assumption in this study as well, but the lack of reaching steady state concentration in the gills made it difficult to support this hypothesis.

The natural uptake of U is predicted to be dynamic throughout the year and through the different seasons. A higher temperature will likely increase the metabolism in the fish, which further increases the uptake and excretion. The fish eats more due to the increased metabolism. If the feed is contaminated, an increased accumulation of U will likely be seen in the stomach, especially if the stomach is not emptied pre-analysis. Waterborne exposures in freshwater are likely more constant throughout the year, but also highly affected by the pH and bioavailability of the species. The pH may have some variation during the year. Erosion due to heavy rain and melting of snow increase the river transport of particles and DOC in the river. This experiment demonstrates that uranium sorbs quickly to food particles, which further decrease the bioavailable fraction.

4. Conclusion

Three hypotheses were tested in this study:

(1) There is an uptake of uranium in the fish direct from water **(supported);**

(2) The concentrations of uranium in gills is higher than in stomach at steady state in aqueous exposure (**not possible to support or contradict);**

(3) Uptake-rates in gills and skin, compared to muscle, kidney and liver are higher **(supported).**

The overall results of this study supported the hypothesis in which an uptake of U directly from water occurs. Uptake of uranium through aqueous exposure was documented by the concentrations in internal organs (muscle, liver and kidney) with no direct contact with the contaminated water. The second hypothesis was not possible to support or contradict, because the uptake in the gills did not reach the estimated concentration of steady state. The third hypothesis was supported, the uptake-rates in gills and skin were higher, compared to muscle, kidney and liver.

The highest concentration of U was detected in the gills. The stomach (w/content) showed the second highest U concentration and reached estimated concentration of steady state. The kidney had a significantly lower U concentration than the stomach. The accumulation of U per weight tissue was higher in the gills than stomach w/content. The uptake-rate was higher in the gills than in all the other organs analysed. The main goal of the experiment was to identify the uptake-rates of U in fish using juvenile Atlantic salmon (*Salmo salar)* as a model organism, which was not possible because the requirements in hypothesis two were not met.

The concentration of the LMM U-species changed in the water over time, most likely due to sorption of U to food particles. This was also supported with the fact that the fraction of U colloidal and particles increased with time. Results demonstrated that it was essential to perform fractionation of U species and at several timepoints during exposure experiments as U can be present in different U species, and the distribution can change largely during the experimental time periods.

Further work

More information on how uranium accumulates in the fish, depending on U species and available DOM, is needed to fully understand how the element is transferred in the ecosystem.

The main pathway for uptake of uranium remains to be determined, and the biological half-life of uranium in Atlantic salmon (*S. salar)* is still scarce. Feeding fish with contaminated food and compare with waterborne exposure can identify the main uptake pathway.

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Appendix

Appendix 1: U distribution in fish species (Kazakhstan, Tajikistan and Kyrgyzstan)

Table A.1: Overview of quantified U concentrations in different fish species sampled in Kazakhstan, Tajikistan and Kyrgyzstan.

Site	**Fish specie**	**U conc. in tissue (number)**	**U conc. (water)**	**pH**	**Source**
Kazakhstan (Kurday mining site)	*P. Schrenkii*	3.5 ± 1.1 mg/g gill ww (n=23)	1.3 mg/L	8.5	(Salbu et al., 2013; Strømman et al., 2013)
		3.1± 3.4 mg/g liver ww (n=20			
		0.14 mg/g muscle ww (n=12)			
Tajikistan (Taboshar Pit Lake)	*C. Auratus*	9.1 mg/kg kidney ww (n=13)	1.4 mg/L	8	(Skipperud et al., 2013b)
		14 ± 29 mg/kg liver ww (n=13)			
		8.9 mg/kg gill ww (n=13)			
		0.3 ± 0.1 mg/kg muscle ww (n=13)			
Kyrgyzstan (Kadji Sai)	*L. Bergi, P. Schrenkli, O. Mykiss*	0.068 ± 0.1 mg/kg gill ww (n=11), 0.025 mg/kg gill ww (n=2), 0.077 ± 0.1 mg/kg gill ww (n=4)	41 µg/L	7.6-8.7	(Lind et al., 2013)
		0.079 ± 0.05 mg/kg liver ww (n=7), 0.010 mg/kg liver ww (n=2), 0.012 ± 0.005 mg/kg liver ww (n=5)			
		0.0053 ± 0.004 mg/kg muscle ww (n=10), 0.0017 mg/kg muscle ww (n=1), 0.0017 mg/kg muscle ww (n=1)			

Appendix 2: Details of analysis and calculations examples

Standards for the water samples

Three standards and wash were prepared before running the ICP-MS. Four 50 mL-tubes were half-filled with distilled water before 5mL UP HNO_3 was added. The concentrations of standard 3 (100 µg/L) is listed in **table A.2.** The different reagents were added. The volume was diluted to 50mL. Five milliliters (10 %) was transferred to standard 2 (10 µg/L), before this tube was diluted to 50mL. The same process was repeated to make standard 1 (1 µg/L). Standard 0 consisted only of acid and distilled water.

Table A.2: Concentrations of standard 3.

Element	Concentration
Na	50 mg/L
Mg	10 mg/L
K	5 mg/L
Ca	30 mg/L
U	100 /L
P	5 mg/L
S	5 mg/L

Ten blank tests were prepared. They consisted of RO-water and 1.3 mL UP HNO_3. A wash-solution was made with 250 mL bottle filled with distilled water and 25 mL UP HNO_3. A certified reference material (1640a) and a house standard (1643H) were used to measure the accuracy of the instrument. The instrumental error of ICP-MS was <2 %, after measuring the different samples.

Internal standard

The internal standard contained indium (In), bismuth (Bi) and rhodium (Rh), and a 5 %-concentration of HNO_3. The Bi was used as internal standard for medium molar mass-elements like uranium, while In and Rh were used as internal standard to low molecular mass-elements like Na and Ca. The final concentration of internal standard in the sample, after dilution, was 2 µg/L.

Standards for fish tissue

Standards and online standard were made in advance. Four 50mL-tubes were half-filled with distilled water, before added 5 mL of UP HNO_3. The different elements were added to standard 2 by using diluted certified standards. Concentrations of the different elements are listed in **table A.3.** Internal standard (1 mL) were added before diluting the sample to 50mL. The fourth tube was used for the online standard with Thorium (Th). First 30 % EtOH was added to the tube before the distilled water. EtOH was added to keep the standard stable in the

ICP-MS and by having a more similar matrix to the samples. Five milliliters of UP HNO_3 was added. The final step was to add the Th (20µg/L).

Table A.3: Concentration of different elements in the ICP-standards. A description of the different elements and their concentrations in standard 1, standard 2 and standard 0. A column provides the volume of each element added to Standard 2, and the original concentrations of the elements.

Element	**St2 (Concentration) 100**	**Added volume St2**	**St1(Concentration) 10**	**St0(concentration) 0**
Na	20 mg/L	0.1 mL (10000mg/L)	2 mg/L	0
P	100 mg/L	0.5mL (10000mg/L)	10 mg/L	0
S	100 mg/L	0.5 mL (10000mg/L)	10 mg/L	0
K	50 mg/L	0.250 mL (10000mg/L)	5 mg/L	0
Ca	50 mg/L	0.250 mL (10000mg/L)	5 mg/L	0
Rb	50 µg/L	0.250 mL (10mg/L)	5 µg/L	0
Sr	100 µg/L	0.5 mL (10mg/L)	10 µg/L	0
V	20 µg/L	0.1 mL (10mg/L)	2 µg/L	0

It was prepared certified reference material, 25 blank tests and wash-solution (250 mL bottles, 25 mL UP HNO3). The blank tests consisted of UP HNO3, distilled water and internal standard with the same concentrations as the fish samples.

Moderate hard EPA

Salts:

- $CaSO_4*2H_2O$
- $MgSO_4$
- KCl
- $NaHCO_3$

Drift (Terum, 2019):

$$3. K = \frac{Std\ 2\ (start)}{\frac{Std\ 2\ (start) + Std\ 2\ (middle)}{2}}$$

Bias (%) (Terum, 2019):

$$4. \frac{Measured\ value - True\ value}{True\ value}\ x\ 100\% =$$

Average and standard deviation, (example with zinc) (Terum, 2019):

$$5. = Average\ (U\ in\ kidney\ samples)$$
$$6. = STDEV(U\ in\ kidney\ samples)$$

% relative standard deviation (example with U in kidney) (Terum, 2019):

$$7.\ \%RSD = \frac{standard\ deviation\ (U\ in\ kidney)}{average\ (U\ in\ kidney)} * 100\%$$

LOD and LOQ (example with U) (Terum, 2019):

$$8.\ \ LOD = 3 * STDEV(blank\ samples: U)$$

$$9.\ LOQ = 10 * STDEV\ (blank\ samples: U)$$

Appendix 3: Uptake rate

Calculations of uptake rate (example gill)

$$F(x) = 30\ 298.18 \text{ ng U/g } (1\text{-}e^{-0.008x})$$

$$\frac{30\ 298.18}{1000} * 0.008 = 0.242384$$

$$= 0.24 \frac{\mu g\ U}{g\ tissue}\ per\ day$$

Appendix 4: Calculation of correlation factor

Example gill and kidney (day 28): The concentrations in gills and kidney in the same fish were divided. The average factor, was then calculated:

$$average(\frac{U\ conc: gill}{U\ conc.: kidney})$$

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