Akvaplan niva

Rapport Report

Risk assessment and risk reducing measures for discharges of hydrogen peroxide (H_2O_2)

Ecotoxicological tests, modelling and SSD curve

Oceanographic modelling

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Summary

This project has provided a tool combining dispersal modelling with ecotoxicological data such as LC_{50}, EC_{50}, NEC and PNEC to determine the area of potential impact following de-lousing with H_2O_2. There was a great variation in the sensitivity towards H_2O_2 for the species tested, however, effects occurred at concentrations well below the concentrations normally used in the fish cage and discharged to the environment. Dispersal modelling shows that relatively high concentrations of H_2O_2 can occur close to the farm and potentially affect the ecosystem. Diluted concentrations, which can affect some species, will spread further away from the release site. The size of the influence area can vary due to variable currents, wind and stratification, as well with species diversity. We conclude that there is a risk for impacts on local ecosystems. However, the potential impacts on a larger scale is still not known. There is a need for combining ecological, ecotoxicological and dispersal modeling to evaluate risk at a larger scale.

The modelling shows that using a wellboat will reduce the environmental impact substantially, but there is still risk for harmful concentrations for some species.

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In addition to Akvaplan-niva, Nouryon has copyright to chapter 4 (ecotoxicology) in this report.

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Preface

In this project, environmental risks of hydrogen peroxide (H_2O_2), used in bath treatments against sea lice, have been assessed.

The project is a continuation of the project "Environmental risk using hydrogen peroxide. Ecotoxicological assessment and limit value for effect" (FHF project no. 901249). The work in this project has focused on ecotoxicological experiments and modelling, and oceanographic modelling. Then risk assessments have been performed based on the modelling results.

The ecotoxicological work has been performed by Akvaplan-niva and Institute for Marine Research (IMR). All oceanographic modelling and risk assessment have been done by Akvaplan-niva. Two industrial partners, Aqua Pharma and Sølvtrans, have provided input on use and routines for administration and release of H_2O_2 to ensure that the performed work was based on realistic scenarios. Nouryon has contributed to the generation of a species sensitivity curve.

Bjørn Munro Jenssen, Norwegian University of Science and Technology (NTNU) and Jarle Berntsen, University of Bergen, has constituted the reference group for the project. We wish to thank the industry partners and the reference group for valuable input to the project.

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Tromsø 01.09.2019

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1 Summary

Hydrogen peroxide (H_2O_2) is used as a delousing agent in the Norwegian aquaculture industry to combat salmon louse (*Lepeophtheirus salmonis*). Recent studies have revealed that effects on non-target species may occur at highly diluted treatment concentrations, even after short exposure times. Hence, risk assessment approaches for marine environments are requested to manage potential impacts associated with release of salmon lice pesticides from the aquaculture industry. In the current project, an internationally recognized environmental risk assessment tool has been used to make an objective assessment of the effects of H_2O_2. Ecotoxicological test have been performed for important ecological and commercial species from Norwegian marine ecosystems, and ecotoxicological metrics have been generated. A species sensitivity distribution curve (SSD) was created in order to define the threshold value for effects on communities; the predicted no effects concentration (PNEC). The PNEC for intermittent releases of H_2O_2 is estimated to 0.14 mg/L.

Oceanographic modeling in 3-D was performed to assess predicted environmental concentrations (PEC) of H_2O_2 in the environment after releases from cages and wellboats. H_2O_2 is denser than water, and the density of the mixture of seawater and H_2O_2 used for delousing is about 1-2 per mill larger than the surrounding seawater. In a weakly stratified water mass, this leads to a rapid sinking after release. The sinking will occur within a few minutes after release and has an important impact on the horizontal spreading of H_2O_2. Thus, modelling in 3-D is necessary to give a realistic picture of dispersal in the environment.

Releases from a site with four cages (one cages deloused at a time, all four deloused over 2 days) were compared to releases from a single cage, and releases from cages of 160 m circumference were compared to releases from cages of 120 m circumference. Local conditions, such as water depth, currents, stratification etc. in the release area will affect dispersal and dilution. Therefore, modelling has been performed for four different areas, representing a range of environmental conditions.

The oceanographic modelling show that concentrations exceeding PNEC can persist in the environment for several hours after release. The duration of exposure above PNEC is of course longer after a 4-cage delousing operation than after a single cage case. A general observation from all four model locations is that concentrations up to about 300 mg/l can occur up to about 1 km from the release site, while 10 mg/l can occur ~5km from the release. Release from a 160 m fish cage will give higher concentrations and a larger affected area than release from a 120 m cage. Since the minimum amount of time between releases in the 4 cage experiments is 6 hours, the effect of the previous release on the next is on average little, but for low concentrations it can still make a difference (overlap may increase concentrations).

Delousing in a wellboat and subsequent release of H_2O_2 yields far lower concentrations in the water masses than releases from cages. Also, the average hours with concentrations above PNEC is much lower for the wellboat release. The results therefore indicate that the impacts from wellboat releases are considerably lower than the impact of direct cage releases.

In the last phase of the project, ecotoxicological metrics, such as PNEC and LC_{50} (concentration that causes mortality of 50% of a group of test animals in a specified period) and NEC (No Effect Concentration), for individual species were compared to the PEC (Predicted Environmental Concentration) values for the selected discharge scenarios at all four different geographical locations. For the different species tested there was large variation in the sensitivity to H_2O_2, but mortality and sub-lethal effects generally occurred at concentrations

much lower than the normal treatment concentrations. Results from the risk assessment comparing PEC to PNEC, revealed that negative environmental effects are likely to occur after H_2O_2 is discharged into the environment. The primary producers, forming the basis for the food chains, are particularly sensitive, and algae can be affected several kilometers away from the discharges. Crustaceans were slightly more robust than algae. Intertidal amphipods and edible crab were shown to be very robust to H_2O_2.

As threshold level for effects are exceeded, the results suggest that biological communities are not protected after discharge of H_2O_2. However, the risk varies among the different species tested and among the different geographical test locations. The risk is reduced when a wellboat is used. Concentrations associated with mortality of a number of non-target species will still be present when wellboats are used, but within a much smaller area compared to discharges directly from the cage and only for a very short time. Wellboats can move to areas away from areas with aggregations of sensitive species prior to releases of chemicals. Using a wellboat is therefore the most important risk reducing measure defined in the current project.

This project has provided a tool combining dispersal modelling with toxicological data as LC_{50}, EC_{50} and NEC to determine the area of potential impact following de-lousing with H_2O_2. The modelling shows that relatively high concentrations of H_2O_2 can occur close to the farm and potentially affect the biological communities. Potentially harmful lower concentrations can spread several kilometres away from the release site where the most sensitive species may be affected. The size of the influence area can vary due to differences currents, wind and stratification, as well as occurrence of sensitive species.

The results show that effects on local communities are likely to occur after releases of H_2O_2. Since the dynamics in ecosystems are not fully understood, it is complicated to extrapolate impacts to larger areas, e.g. Norwegian coastal systems. Compensating mechanisms or cascading effects are difficult to predict. Thus, in the future, ecological, ecotoxicological and dispersal models should be combined in order to estimate total, long-term risks related to releases of delousing chemicals. A challenge that remains for ecotoxicologists and policymakers is to define what effects and risk are acceptable (or unacceptable) related to the use of delousing chemicals.

Main findings:

• The Predicted No Effect Concentration (PNEC) for intermittent releases of H_2O_2, i.e. the concentration that is not expected to cause harm to biological communities, is calculated at 0.14 mg/L.

• The sensitivity varies between different animal groups, species and life stages. Algae are most sensitive to H_2O_2, followed by crustaceans. Fish have the lowest sensitivity.

• After releases from cages, concentrations of H_2O_2 exceeding PNEC can persist in the water column for several hours. This means that H_2O_2 is present long enough to affect a number of species.

• When released from cages, concentrations up to approx. 300 mg/L occur approx. 1 km from the release site, while 10 mg/L may occur ~ 5 km from the release site. In areas where the water masses are not stratified, sinking to the bottom will occur within minutes after release.

• The risk to biological communities is considerably lower when H_2O_2 is released from a wellboat than when it is released from cages.

2 Sammendrag

Hydrogenperoksid (H_2O_2) brukes i norsk havbruksnæring for å bekjempe lakselus (*Lepeophtheirus salmonis*). Nye studier har vist at H_2O_2, i sterkt fortynnede konsentrasjoner, kan påvirke arter som finnes i miljøet rundt oppdrettsanlegg. Det er derfor behov for verktøy som kan gi informasjon om mulig miljørisiko ved bruk av lusemidler. I dette prosjektet har et internasjonalt anerkjent verktøy for miljørisikovurdering blitt brukt for å gi en objektiv vurdering av risiko ved bruk av H_2O_2. Økotoksikologiske tester er gjennomført for økologisk og kommersielt viktige norske arter, og økotoksikologiske parametre er fastsatt. En "Species Sensitivity Distribution" (SSD) kurve er generert for å definere terskelverdien for effekter på biologiske samfunn; "Predicted no effect concentration" (PNEC). PNEC for periodiske utslipp av H_2O_2 ble beregnet til 0,14 mg/L.

3-D oseanografisk modellering ble utført for å beregne konsentrasjoner (PEC – "Predicted Environmental Concentrations") av H_2O_2 i miljøet etter utslipp fra merd og brønnbåt. H_2O_2 har en høyere tetthet enn sjøvann, slik at tettheten til blandingen av sjøvann og H_2O_2 brukt til avlusing er omtrent 1-2 promille høyere enn tettheten til sjøvannet blandingen slippes ut i. I en svakt lagdelt vannmasse synker derfor blandingen raskt. Synking vil skje i løpet av få minutter etter frigjøring og har en viktig innvirkning på spredningen av H_2O_2. Dermed er modellering i 3-D nødvendig for å gi et realistisk bilde av spredning i miljøet.

Utslipp fra et anlegg med fire merder (en merd avluset om gangen, alle fire avluset over 2 dager) ble sammenlignet med utslipp fra en enkelt merd, og utslipp fra merd med 160 m omkrets ble sammenlignet med utslipp fra merd med 120 m omkrets. Lokale forhold, for eksempel vanndybde, strømmer, lagdeling mm. i utslippsområdet vil påvirke spredning og fortynning. Derfor er modellering utført for fire forskjellige områder, som representerer et spekter av ulike miljøforhold.

Den oseanografiske modelleringen viser at konsentrasjoner som overstiger PNEC kan vedvare i flere timer etter utslipp. Konsentrasjoner over PNEC vil naturlig nok vedvare lengre etter utslipp fra 4 merder enn ved utslipp fra en merd. En generell observasjon fra alle de fire modellstedene er at konsentrasjoner opp til ca. 300 mg/L kan forekomme ca. 1 km fra utslippsstedet, mens 10 mg/L kan forekomme ~ 5 km fra utslippet. Avlusning i en merd på 160 m gir høyere konsentrasjoner og et større påvirket areal enn avlusning i en 120 m merd. I beregningene gjennomført for anlegg med 4 merder var minimumsperioden mellom utslipp fra hver merd 6 timer. Fortynning skjer relativt raskt, slik at det blir lite overlapp mellom plumene fra påfølgende utslipp. For lave konsentrasjoner vil overlapp likevel kunne forekomme (overlappende plumer fører til høyere konsentrasjoner).

Avlusing i brønnbåt med etterfølgende utslipp av H_2O_2 gir langt lavere konsentrasjoner i vannmassene enn utslipp fra merd. Konsentrasjoner som overskrider PNEC vil dessuten være tilstede i mye kortere tid enn ved utslipp fra merd. Resultatene indikerer dermed at miljøeffekter vil være betydelig mindre ved bruk av brønnbåt til avlusning enn ved utslipp fra merd.

I den siste fasen av prosjektet, ble økotoksikologiske parametre, som PNEC, samt LC_{50} og NEC for enkeltarter, sammenlignet med PEC-verdiene for de valgte utslippsscenariene. Det var store forskjeller i følsomhet mellom de ulike artene som ble testet, men grenseverdier for effekter (dødelighet og sub-letale effekter) var for de fleste artene betydelig lavere enn behandlingskonsentrasjon (dvs. konsentrasjon som brukes i merd/brønnbåt). Resultater fra risikovurderingen som sammenlignet PEC med PNEC, viste at negative miljøeffekter sannsynligvis vil oppstå etter at H_2O_2 er sluppet ut i miljøet. Alger, som danner grunnlaget for

næringskjeden, er spesielt følsomme, og alger kan påvirkes flere kilometer unna utslippene. Også krepsdyr er følsomme, men noe mer robuste enn alger. Littorale amfipoder og taskekrabbe hadde lav følsomhet overfor H_2O_2.

Resultatene fra risikovurderingen viser at biologiske samfunn kan bli påvirket etter utslipp av H_2O_2. Imidlertid varierer risikoen mellom de forskjellige artene som er testet og mellom geografiske lokasjoner. Risikoen reduseres når brønnbåt brukes. Konsentrasjoner assosiert med dødelighet for en rekke arter, vil fortsatt være til stede etter utslipp fra brønnbåt, men innenfor et mye mindre område enn ved utslipp fra merd og bare i en kort periode. Brønnbåter kan dessuten bevege seg bort fra områder med sensitive arter før utslipp av behandlingsløsning. Bruk av brønnbåt er derfor det viktigste risikoreduserende tiltaket som er definert i prosjektet.

Prosjektet har utviklet et verktøy som kombinerer spredningsmodellering med toksikologiske data, som LC_{50}, EC_{50} og PNEC, for å bestemme areal og volum som kan påvirkes etter avlusing med H_2O_2. Modelleringen viser at relativt høye konsentrasjoner av H_2O_2 kan forekomme nær utslippsstedet, og dette vil sannsynligvis kunne føre til negative effekter for økosystemet. Fortynnede konsentrasjoner spres flere kilometer unna utslippspunktet. Hvor stort område som påvirkes vil variere med strømforhold, vind, lagdeling, samt artsmangfold.

Resultatene viser at det sannsynligvis vil oppstå effekter på lokale samfunn etter utslipp av H_2O_2. Siden dynamikken i økosystemer ikke er helt forstått, er det komplisert å ekstrapolere påvirkninger til større områder, f.eks. hele norskekysten. Kompensasjons-mekanismer eller kaskade-virkninger er vanskelig å forutsi. I fremtiden bør økologiske, økotoksikologiske og spredningsmodeller kombineres for å estimere total, langsiktig risiko relatert til utslipp av lusemidler. Det er dessuten viktig å definere hvilke effekter og hvor høy risiko som er akseptabel (eller uakseptable) ved bruk av avlusingskjemikalier (akseptkriterier).

Hovedfunn:

- PNEC for periodiske utslipp av H_2O_2, dvs. den konsentrasjonen som ikke antas å føre til skade for biologiske samfunn, er beregnet til 0,14 mg/L.
- Det er stor variasjon i sensitivitet mellom ulike dyregrupper, arter og livsstadier. Alger er mest sensitive for H_2O_2, etterfulgt av krepsdyr. Fisk er mest hardfør.
- Etter utslipp fra merd kan konsentrasjoner av H_2O_2 som overstiger PNEC vedvare i vannsøylen i flere timer. Dette betyr at H_2O_2 er lenge nok tilstede til at en rekke arter kan påvirkes negativt.
- Ved utslipp fra merd kan konsentrasjoner opp til ca. 300 mg/L forekomme ca. 1 km fra utslippsstedet, mens 10 mg/L kan forekomme ~ 5 km fra utslippet. I områder hvor vanmassene ikke er lagdelt vil synking til bunn skje i løpet av minutter etter utslipp.
- Risiko for biologiske samfunn er betydelig lavere når H_2O_2 slippes ut fra brønnbåt enn ved utslipp fra merd.

3 Introduction

Aquaculture activities are expanding in Norwegian marine areas. One of the major challenges in aquaculture is the infestation of salmon by the salmon louse *(Lepeophtheirus salmonis)* (Torrisen et al. 2013). Salmon lice are small marine ectoparasites feeding on mucus, blood and skin of salmonids and if present in sufficient numbers they can cause significant damages to the farmed fish. To combat salmon lice, the industry is using several techniques, including different mechanical (e.g. hot water, pressure) biological (e.g. cleaner fish) and chemical treatments. In recent years mechanical and biological treatment have increased, but still many farms use chemotherapeutic treatments (e.g. organophosphates, pyrethroids, and hydrogen peroxide (H_2O_2)) or in-feed treatments (e.g. emamectin benzoate) to keep the sea lice numbers below the allowed levels (Remen & Sæther 2018). The use of H_2O_2 to combat sea lice have increased, from 2009 to 2015 (www.fhi.no; Remen and Sæther 2018), but in later years the use has decreased. The use of H_2O_2 was 9 277 tons in Norway in 2017. In the period 2009-2017, a total of 128 million kg of (100%) H_2O_2 was used (Folkehelseinstituttet, 2018). However, in 2017 the usage was 1/3 compared to 2016 (based on number of treatments doses). In 2018 H_2O_2 was the second most commonly used delousing agent in Norway (Remen 2019). Updated information about the use of chemicals in Norwegian aquaculture is available on the web page of the Norwegian Institute of Public Health.

When the chemicals used to combat sea-lice are released to the surrounding marine environments, negative effects may occur. Negative effects of delousing chemicals for many non-target species have been documented, mainly in laboratory studies (Burridge & Van Geest 2014, Brokke 2015, Refseth et al. 2016, Bechmann et al. 2019). In a recent review by Urbina et al. (2019), an extensive evaluation of published lethal and sublethal effects of delousing chemicals on non-target crustaceans and bivalves was performed. The review showed that negative effects may occur, at concentrations lower than those used in treatments against sea lice, in all species studied. Increased focus on potential negative environmental effects of chemicals after release to the environments calls for development of risk assessment procedures to ensure safe operations and reduce conflict between aquaculture and other industries, e.g. fisheries.

H_2O_2 is considered the most environmentally friendly alternative among the therapeutic agents, as it breaks down to water (H_2O) and oxygen (O_2). Most countries, including Norway do not have stringent regulations for H_2O_2. However, H_2O_2 is more active than molecular oxygen, and it is a strong oxidizing agent. The mechanism of action is lipid peroxidation of cellular membranes and mechanical paralysis, in addition to inactivation of enzymes and DNA replication (Cotran et al. 1989). Toxicity to various organisms has been documented, also at short term exposure to highly diluted treatments concentrations (Van Geest et al. 2014, Fagereng 2016, Bechmann et al. 2019, Haugland et al. 2019). Given the documented negative effects on marine organisms at environmentally realistic concentrations, undesirable effects of H_2O_2 can occur in the environment when released to the surrounding environments. Recent modelling studies show that the substance can persist in the local environment in concentrations that can cause toxic effects on the marine life. The spread does not only occur on the surface, as H_2O_2 will sink relatively quickly through the water column when water masses are mixed (Refseth et al. 2016). Therefore, sinking must be considered in models and risk evaluation.

Risk assessment is a tool used to characterize and quantify risks and to ensure protection of the environment. Today's risk assessment procedures include various metrics of species tolerance to chemical exposures that allow operators to characterize their potential environmental impacts

(Chapman 1995, Forbes & Calow 2002, Calow & Forbes 2003). Survival, quantified through standardized laboratory toxicity tests, is the most widely used expression of species tolerance to chemical exposures (Newman & Dixon 1996). The most common testing protocol is performed by exposing biota to several different concentrations of chemicals. Metrics of survival derived from the toxicity test protocol and applied in risk assessments are concentrations causing lethality to 50% of exposed individuals (LC_{50}) and no-effect concentrations (NEC). NEC is often the starting point for environmental policy. EU Technical Guidance Document of risk assessment (ETG) (EC 2003) and US-EPA Guidance on risk assessment present internationally accepted and recommended methods for risk assessment. In ETG the "predicted no effect concentration" (PNEC) is compared to the "predicted environmental concentration" (PEC) of the chemical of concern. The PNEC is normally calculated from toxicity tests. Different species have different sensitivities to a chemical. This variation can be described with a statistical or empirical distribution function, and this yields a species sensitivity distribution (SSD). The SSD provides sensitivity assessments for a whole community (Kooijman 1987). Scientists use SSDs for the derivation of environmental quality criteria, challenged by policy makers to make optimal use of single-species toxicity test data for chemicals. The SSD approach is now frequently used in environmental management. When less data is available, PNEC can be calculated for individual species. PNEC is then calculated for individual species with an assessment factor to the ecotoxicological metrics.

PEC is normally calculated using oceanographic and chemical fate modelling. To obtain a good picture of distribution in Norwegian coastal areas, with narrow straits and complicated topography, high-resolution modelling is required. Therefore, the circulation model FVCOM (Finite Volume Community Ocean Model, Chen et al. 2003, 2006) has been used for the determination of PEC. FVCOM has an unstructured grid that makes it possible to vary the model resolution in the model domain. This is especially useful when modeling dispersal from aquaculture cages, as it is important to have high resolution around the discharge point. In a previous project, Akvaplan-niva (Apn) and IMR, performed several ecotoxicological experiments with species that are important in Norwegian coastal areas to assess the toxicity of H_2O_2. In addition, oceanographical modelling was performed to estimate environmental concentrations in Norwegian fjords after delousing in a closed tarpaulin. The oceanographic modelling showed that H_2O_2 will sink to the seafloor within a few minutes after discharge if water masses are mixed (mainly during winter season). This was considered by starting the model with a vertical distribution of H_2O_2 calculated using a theoretical model for sinking (Refseth et al. 2016). However, sinking will also play an important role for the horizontal distribution as currents vary between different depths. Therefore, it was important to develop a module in the hydrodynamic model, FVCOM, which can directly model H_2O_2 dispersion in three dimensions.

As a last step in risk assessment the ecotoxicological metrics were compared to the PEC values. If PEC/PNEC value is below 1, it is assumed that risk to the environment is minimal, and no further actions are requested. If PEC/PNEC ratio is above 1, effects are expected, and further investigations and risk reducing measures should be considered, or discharges reduced.

The former project revealed that there was a risk for negative effects for some species after H_2O_2 discharges when comparing results from the oceanographic modeling and the ecotoxicological metrics. However, there was not enough data to establish an SSD curve and to calculate a PNEC value. To asses ecotoxicological risk, an SSD was therefore established in the current project.

3.1 Aims

During recent years numerous studies on effects of chemicals used in de-lousing operations have been conducted. However, the potential effects on non-target species inhabiting areas where salmon farming develops may not be well represented considering that these effects are species-specific. It is therefore necessary to evaluate the effects of these delousing chemicals at the local level, using local species, representatives of the zones that are subject to the impacts of these delousing chemicals in the marine environment (Urbina et al. 2019).

In the present study we used the principles in risk assessment to assess risk related to H_2O_2-discharges from aquaculture industry in Norway. Derived species tolerance values were compared to modelled concentrations in the environment. The results were assessed, and applicability of the method discussed, in accordance to today's practise. In addition, different risk-reducing measures has been explored.

The aim of the project was to assess the environmental risk related to discharges of H_2O_2 used as delousing agent in the aquaculture industry, as well as to suggest risk reducing measures.

The following sub goals were set:

- To establish a Species Sensitivity Distribution (SSD) curve and PNEC-values for H_2O_2.
- To develop a 3D dispersion model for direct simulation of sinking and spreading of H_2O_2.
- To simulate dispersions from cages and wellboats with the 3D model, using chosen discharge scenarios defined in collaborations with the industry partners.
- To assess risk to ecosystem for the different scenarios, by using an international accepted risk assessment method.
- To suggest feasible risk reducing measures, in collaboration with the industry partners.

4 Ecotoxicology

4.1 Background

Risk assessment approaches for marine environments are requested to manage potential impacts associated with release of salmon lice pesticides from the aquaculture industry. Ecotoxicological metrics such as LC_{50}, NEC and PNEC are often used in risk assessment, and sufficient data for different ecosystem components are needed in order to evaluate risk to communities and ecosystems. In this project toxicity tests of H_2O_2 on marine species were performed, and additional ecotoxicological data were collected from literature and reports. The experimental results from laboratory tests were processed in a biological based model and used to quantify ecotoxicological metrics. The ecotoxicological metrics were integrated in an overall SSD-curve to calculate the PNEC concentration. Finally, the ecotoxicological results (chapter 4) were compared to the results from oceanographic modelling (chapter 6) in the risk assessment performed in chapter 7.

4.2 Material and methods

To generate ecotoxicological metrics for use in risk assessment, toxicity tests were performed on selected species covering important functional and taxonomic groups from Norwegian marine ecosystems. The experiments were performed by Apn and IMR. The selected species are described in chapter 4.2.1, and experimental set-up for the species tested are presented in chapter 4.2.2. Thereafter, a description of ecotoxicological modelling, performed on raw data from the experiments, is provided in chapter 4.2.3. The last section describes the methodology used for generating SSD curves (chapter 4.2.4). Finally, the results are presented and discussed in chapter 4.3.

4.2.1 Species

Ecologically and commercially important species from Norwegian marine ecosystems were chosen for the ecotoxicological experiments. To investigate potential differences in sensitivity between taxonomic groups and to ensure ecological relevance, species were selected from different taxonomic and functional groups, as well as from different habitats. Species selected for laboratory experiments in the present study were green sea urchin (*Strongylocentrotus droebachiensis*), common whelk (*Buccinum undatum*), amphipoda (Gammarus sp., very likely *Gammarus locustraunde*)), edible crab (*Cancer pagurus*), sugar kelp (*Saccharina latissima*), and the polychaetes *Capitella sp.* and *Ophryotrocha spp*. These species were exposed to H_2O_2 and are described more in detail below. Furthermore, results for species exposed to H_2O_2 in a previous project (Refseth et al. 2016) (Table 1) are also presented here, since these data were integrated in the overall SSD curve (*Cyclopterus lumpus*, *Pandalus borealis*, *Gadus morhua*, *Calanus finmarchicus*, *Palaemon elegans* and *Praunus flexuosus*).

Green sea urchin (*Strongylocentrotus droebachiensis*) is among the most common species along the Norwegian coast (Moen & Svensen 2008). Green sea urchin is an important ecological species, as it is frequently found in the diet of several species, such as crabs, fish and mammals.

Common whelk (*Buccinum undatum*) is a common marine species in the North Atlantic, and along the coast of Norway, and other northern European countries. This species lives in the sublittoral and littoral zone, on sand and soft bottom down to 100 meters depth.

Amphipoda (Gammarus sp., very likely *Gammarus locustraunde*) are important keystone species in aquatic ecosystems because of their role in the detritus cycle. In addition, they represent an important element in food webs by providing prey for secondary consumers (Bulnheim 1979). They typically occur from the low intertidal to about 30 meters depth.

Edible crab (*Cancer pagurus*) is commonly found on bedrock, under boulders, on mixed coarse grounds, and offshore in muddy sand. It typically occurs from shallow sublittoral to offshore to about 100 meters depth. It is an active predator and consumes a variety of crustaceans, and cannibalism on smaller members of their own species (www.marlin.ac.uk/species/detail/1179).

Sugar kelp (*Saccharina latissima*). Along the Norwegian coastline, one of the most dominating macroalgal habitats are kelp forests. Kelp-species are important primary producers and key components in coastal ecosystems. On sheltered and medium-exposed locations, the sub-littoral vegetation is commonly dominated by the perennial sugar kelp (Moy & Christie 2012). Kelp forests are biodiversity hotspots, serving key functions in the ecosystem by providing refuge, habitat, nursery grounds, and feeding grounds for >100 marine faunal species, including economically important fish species, such as Atlantic cod, saithe and seabirds.

Polychaetes such as *Capitellla sp. and Ophryotrocha spp*. are common in benthic habitats under fish farms and in other types of anthropogenically modified estuaries (Bannister et al. 2014). Opportunistic polychaetes that are adapted to nutrient-rich habitats and commonly found underneath fish farms located over hard bottom in Norway include *Vigtorniella ardabilia* and over soft-sediment areas *Ophryotrocha* spp. The polychaetes are important for environmental recovery as they consume and transform organic materials deposited from the fish farms (Dean 2008). These species live near the fish farms, and they may therefore be exposed to agents originating from activities at the farm, including salmon lice treatment.

The following species were tested in Refseth el. (2016):

Lumpfish *(Cyclopterus lumpus)* is a common fish species along the entire Norwegian coast. The lumpfish is harvested in Norway because of the roe, which is used in caviar production. In the spring, lumpfish arrive at the coast to spawn in shallow water. The first two years the juveniles are located in the beach zone, and later on they migrate into deep water. Adult fish live pelagically at 50-150 m deep, and eat pelagic crustaceans and jellyfish (Schopa 1974, Vasconcelos et al. 2004).

Deep sea shrimp (*Pandalus borealis*) lives above the sea bottom, at depths between 50 and 600 m, on a clay bottom. They can also occur as shallow as 15-20 meters. They perform periodic migrations with 24-hour migrations into free water masses (grazing behavior) and yearly to shallower areas (spawning migration). Deep sea prawns spawn in the fall, and the females carry the eggs attached to the hindquarters' flippers until next spring. Larvae hatching from the eggs live pelagically for approximately three months before searching for the bottom. In Norway, deepwater shrimp is the most important commercial crustacean species.

Atlantic cod *(Gadus morhua)* is a fish species in the cod family. It is an important commercial species in Norway. The coastal cod spawns both inside the fjords and in the archipelago. It selects protected areas, often at the bottom of the fjord arms and bays, where spawning typically takes place at 20-60 m deep. The eggs float mostly in the top 30 m of the water column and hatch after 2-3 weeks (information from IMR).

Calanus finmarchicus constitutes the major part of the zooplankton in the ecosystem of the Norwegian Sea and is thus a very important ecological species. *C. finmarchicus* graze on phytoplankton and is an important prey for fish larvae and adult pelagic fish. For many species of fish (larvae), the egg and nauplii of *C. finmarchicus* are the most important food source. *C.*

finmarchicus is a very important biological resource, as the annual production in the North Atlantic is many times higher than the total biomass of all fish species in the same area, including cod, herring and mackerel.

Grass prawns *(Palaemon elegans)* is a common species in the North Sea, the Baltic Sea, the East Atlantic, the Mediterranean and the Black Sea (Ozen & Samsun 2009). The species lives on rocks or sandy sediments in shallow depths in the tidal zone. The grass prawn is a generalist and opportunist with an omnivorous diet and is found in different habitats (Berglund 1980).

Chameleon shrimp (*Praunus flexuosus*) is one of the most common mysid species in Norway. *P. flexuosus* is a pelagic species, living in the upper part of the tidal zone. They are filter feeders and/or predators (feeds on copepods, algae or amphipods) in the tidal zone (Tattersall & Tattersall 1951).

Table 1 lists all species which were included in experiments in the current project and in Refseth et al 2016. For two of the species, edible crab and green sea urchin, ecotoxicological metrics could not be calculated (see 4.3). These species are therefore not included in the overall SSD curve. In addition to the species in Table 1, available data collected from the literature were included in the overall SSD curve. An overview of all species which were included in the curve will be presented later, for algae (Table 6), invertebrates (Table 7) and fish (Table 8).

Table 1. Overview of species tested in ecotoxicological experiments and modelling in the current project and in Refseth et al. 2016. These species are included in the SSD curve, except edible crab and green sea urchin (ecotoxicological metrics could not be calculated for these species).

Species	Taxonomy (Class)	Habitat	Functional group	Institute	Wet weight (g)	Life stage	Temp (°C)	Reference
Lumpfish (C. *lumpus)*	Actinoptyrgii	Pelagic	Predator	APN	75	Juvenile	10.2	
Deepsea shrimp (*P. borealis)*	Malacostraca	Benthic /Pelagic	Predator	APN	20,7	-	10.2	
Green sea urchin (*S. droebachiensis)*	Echinoidea	Benthic	Grazer	APN	65	Adult	10.4	
Common whelk (*B. undatum)*	Gastropoda	Benthic/ Sub-littoral	Predator	APN	2,6	Juvenile	10.3	
Atlantic cod egg (*G. morhua*)	Actinoptyrgii	Pelagic	-	IMR	-	Egg (4 days)		
Copepod (*C. finmarchicus*)	Copepoda	Pelagic	Grazer	IMR	-	Adult	8.0	Escobar Lux 2016
Rockpool shrimp *P. elegans*	Malacostraca	Benthic	Generalist opportunist	IMR	-	Adult	12.9	Brokke 2015
Chameleon shrimp (*P. flexuosus)*	Malacostraca	Pelagic	Filterer predator	IMR	-	Adult	12.9	Brokke 2015
Polychaetes (*Capitella sp.)*	Polychaeta	Benthic	Sediment feeder	IMR	-	-	8-9	Fang et al. 2018
Polychaetes (*Ophryotrocha spp.)*	Polychaeta	Benthic	Sediment feeder	IMR	-	-	8-9	Fang et al. 2018

Sugar kelp (*S. latissima*)	Laminariaceae	Intertidal/ Sub-littoreal	Primary producer	IMR	-	-	8	Haugland et al. 2019
Common whelk (*B. undatum)*	Gastropoda	Benthic/ Sub-littoral	Predator	APN	26	Adult	8-9	
Amphipods (*Gammarus spp.)*	Amphipoda	Intertidal/ Sub-littoral	Generalist, opportunist	APN	-	Juvenile	8-9	
Edible crab (*C. pagurus)*	Malacostraca	Intertidal/ Sub-littoral	Predator	APN	560	Adult	8-9	

4.2.2 Experimental setup

In the following text experiments performed in the present project are described. For a description of experimental animals and experiments performed in the previous project, see material and method section in Refseth et al. (2016).

Akvaplan-niva

Green sea urchins (Figure 1) with an average weight of 65 g were collected in Kvalsundet (69° 45′15.9″N; 19°01′51.4″ W) at 2.5 - 3 m. Urchins were transported to Akvaplan-niva's research station FISK (Forsøks- og Innovasjons Stasjon Kraknes) and held in a 300-liter tank with running sea water (*in situ* temperature) and reduced light. The sea urchins were acclimated for 96 hours before the experiment started.

Figure 1. Green sea urchins. Foto: Gro Harlaug Refseth.

Juvenile common whelks with an average weight of 2.6 g were collected from Kvalsundet (69°45′15.9″ N; 19°01′51.4″ W) at low tide. The snails were acclimated in 45 L tanks (*in situ* temperature) for at least 24 hours prior to initiating the experiment.

Adult common whelk with an average weight of 23 g were collected from Kvalsundet (69°45′ 15.9″N; 19°01′51.4″W). The snails were acclimated in 20 L plastic buckets (*in situ* temperature) for at least 24 hours prior to initiating the experiment. The whelks were not fed before or during the experiment.

Figure 2. Common whelk. Foto: Gjermund Bahr.

Amphipods (Figure 3) were collected from Kvalsundet (69 ° 45 ′ 15.9 ″ N; 19 ° 01 ′ 51.4 ″ W) at low tide. The animals were acclimated in 1 L transparent plastic beakers (*in situ* temperature) for at least 24 hours prior to initiating the experiment.

Figure 3. Amphipod. Foto: Starrlight Augustine

Edible crabs (Figure 4) with an average weight of 2500 g were collected in Hekkingen (69°36'18.0"N 17°49'44.1"E). The specimens were transported to the research station and held in a 12 000 L tank with running sea water (*in situ* temperature) and reduced light. The crabs were acclimated for several days before the experiment started. The crabs were fed before the acclimatization and experiment started.

Figure 4. Edible crab. Foto: https://www.imr.no/temasider/skalldyr/taskekrabbe/nb-no

The exposures were carried out at Akvaplan-niva's research station FISK (Forsøks- og Innovasjons Stasjon Kraknes) in Tromsø. All species were acclimated in plastic buckets or tanks with sand-filtered seawater before the experiments were initiated. For each species, 5 concentrations of H_2O_2 and a control were prepared before the animals were placed in the treatment tanks.

For green sea urchin and edible crab, 60 L tanks filled with 45 L of filtered seawater pumped from an intake of approximately 60 m depth, were used. The selected set up for the 24 h acclimatization was flow-through and water was carefully regulated to a flow of 50 L/h to supply oxygen and to keep the water temperature stable. During the experiment the water flow was turned off to a static exposure throughout the 24 h exposure to achieve a constant concentration of H_2O_2. Oxygen and temperature levels were monitored at the beginning and end of the experiments. The urchins were not fed before or during the experiment. Unfortunately, due to the sea urchin ability to retract it was not possible to estimate mortality during the 24 h exposure.

For amphipods, 1 transparent plastic beakers were filled with 1 L of filtered seawater pumped from an intake of approximately 60 m depth. The selected set up for acclimatization and experiment was a static system. The exposure was performed in a cooled room where the water temperature was stable at around 12 °C throughout the experiment and light was turned off. The amphipods were not fed before or during the experiment.

For green sea urchin and juvenile common whelk, a pilot consisting of four treatments was prepared: 3 doses of H_2O_2 (20, 60 and 100 mg/L) and control. For each treatment, one replicate per concentration was set, giving 4 treatment tanks per species (control included). Each replicate contained 4 individuals of the test species and treatment was only initiated if the animals did not show any signs of stress.

For amphipods and edible crab, five treatments were prepared: 4 doses of H_2O_2 (2000, 4000, 6000 and 8000 mg/L for amphipods; 100, 200, 500 and 1000 mg/L for edible crab) and control. For each treatment, there were 3 replicates for each concentration, representing 15 treatment beakers/tanks per species (control included). Each replicate contained 4 individuals for the edible crab and 10 individuals for the amphipods, and treatment was only initiated if the animals did not show any signs of stress. Unfortunately, due to cannibalism during the exposure and a high tolerance of this species, it was not possible to complete the experiment for this species.

Due to the escape response and to avoid cross-contamination, each amphipod-beaker was covered with a plastic lid which was removed for visual observation of survival at each time point. Mortality was recorded immediately after exposure at eight different timepoints T0, 0.5, 1, 2, 4, 6, 12 and 24 hours after exposure i.e. in the recovery period. To minimize interference, as little light as possible was used for inspection. The activity of the animals was observed, and a reaction provoked to seemingly dead specimens to verify whether the animals were dead or still alive (motion). The dead animals were removed from the exposure tanks at the observation time. Changed behaviour was also noted on the surviving individuals.

Temperature and dissolved oxygen were measured at T0 and T24 in all replicates. H_2O_2-concentrations were measured at T0 and 24 in all replicates, and in a randomly selected replicate from each treatment at T6 and 12 using Abcam's Hydrogen Peroxide Assay Kit (Https://www.chemetrics.com/index.php?route=product/product&product_id=497).

Institute of Marine Research

First-year sugar kelp (*Saccharina latissimi*, Figure 5) was collected in the upper subtidal zone (1− 3 m depth) at Hjellestad, SW of Bergen, Norway (60°15'40.4"N, 5°12'31.7"E) and transported to the Institute of Marine Research, Bergen. Prior to initiation of the experiment, plants were kept at their collection temperature of 8°C in 15 L aquariums for minimum 24 h. Six fluorescent daylight lamps provided irradiance (Haugland et al. 2019). A submersible micropump (flow rate: 150 L $h^{-1)}$ maintained circulation of the water. Seawater in the aquarium was changed every other day, and no growth medium was added. Following lab acclimatization, 5 plants without wounds or fouling were chosen, numbered individually, measured by volume, and randomly assigned to 1 of 5 H_2O_2 exposure concentrations. The selected concentrations were based on a preliminary dose–response study (see Haugland et al. 2019) and ranged from 10% to 0.1% of the bath-treatment dose of 1700 mg/L H_2O_2 recommended by the producer for a temperature of 8°C. Plants were exposed in individual 2 L beakers for 1 h under low (50 PAR) light conditions. A total of 30 plants were included in the main study and divided equally between the 5 H_2O_2 concentrations (i.e. 6 replicate plants per concentration). As the preliminary study indicated that effects may not be apparent until several hours post exposure, incubations to determine the effects on photosynthesis were conducted at 3 post-exposure time points: immediately after exposure (Day 0), 24 h post-exposure (Day 1), and 15 d post-exposure (Day 15), giving a total of 90 individual incubations.

Toxicity potentials, including lethal concentration for 50 % of the population (LC_{50}) and effect concentration (EC_{50}), for photosynthetic capacity (PMAX) and efficiency (α) were determined based on these data (for more details see the material and method section in Haugland et al. 2019).

Figure 5. Saccharina latissimi. Foto: Barbro Taraldset Haugland

Capitella sp. were collected by grab sampling underneath a fish farm located at Austevoll, Norway. *Ophryotrocha spp.* were collected underneath a fish farm in Hardangerfjord, Norway, using artificial plastic grass mounted in an iron frame and deployed underneath a fish cage. At both fish farms, mechanical methods (fresh water, increased water temperature) had recently been used for delousing purposes. Directly after sampling, polychaetes were placed in boxes containing sea water collected from about 150 m depth. The boxes, supplied with air, were transported to the laboratory at Austevoll Research Station (Institute of Marine Research, Norway). Capitella sp. specimens were placed in four 100 L tanks, with 1 kg of glass beads (6 mm diameter) in each tank mimicking artificial benthic substrate. The *Ophryotrocha* spp. were placed in 100 L tanks, each supplied with 5 stones of about 300 g serving as substrate. The stones facilitated aggregation of *Ophryotrocha* spp. and provided a rough substrate to attach mucus strings, mimicking a hard-bottom substrate. Tanks were supplied with a seawater flow of 1150 to 1500 mL/min from 150 m depth holding a temperature of 8 to 9°C. The polychaetes were acclimatized for 5 days and fed ground salmon pellets produced by Skretting once a day. The tanks were kept in darkness during the acclimation period, except during feeding. Polychaetes were exposed to 6 nominal concentrations of H_2O_2 (100, 200, 400, 800, 1200, 1800 mg/L) for 1 h, where the highest concentration is equal to the recommended dose used for treatment. Concentrations were prepared by diluting the stock formulation (Nemona 49, 5%, Nouryon) with sea water to the desired concentration for each treatment. The polychaetes (>50 individuals, estimated from pre-calculated volume per number) were transferred to 2 L beakers containing the decided concentration of H_2O_2. Beakers without H_2O_2 served as controls. Three replicate groups were used for each concentration, including control groups. Following exposure (1 h), the H_2O_2 solution in the beaker was replaced with clean water and a continuous flow (150−180 mL/min) of sea water established. The number of dead animals was recorded at 1, 6, 12, 24, 48 and 72 h from the start of H_2O_2 exposure; the number of remaining survivors was also counted at 72 h. For more details, see Fang et al. (2018).

Figure 6. Capitella capitata. Foto: By © Hans Hillewaert, CC BY-SA 4.0, https://commons.wikimedia.org/w/index.php?curid=398359

4.2.3 Ecotoxicological modelling

NEC and LC_{50} are important ecotoxicological endpoints used in environmental risk assessments. In the current project, the DEBtox model have been used to calculate LC_{50}-values and NEC based on raw data from the laboratory. Below is a description of the LC_{50} and NEC, as well as a description on how the DEBtox model was used to calculate these ecotoxicological metrics.

The LC_{50} is one of several ecotoxicological endpoints that quantify toxicity and it is widely used in ecotoxicological assessments. For a more comprehensive picture of toxicity, values such as No Observed Effect Concentration (NOEC), Lowest Observed Effect Concentration (LOEC) and NEC are also used. NEC is a recommended risk assessment parameter but is less often calculated in ecotoxicological studies compared to LC_{50}. In order to estimate the NEC, one must have access to raw data from laboratory studies, and the dataset must include enough observations over time. These data are not always available in publications. Even with access to raw data, it is not always possible to calculate the NEC, it depends on how the animals respond to the chemical. If most animals die from one point to the next, or if the animals die at the start of the experiment, and there is no even distribution of mortality throughout the experiment, the NEC cannot be calculated. NEC can also not be calculated using DEBtox if significant growth is expected during the experiment (www.debtox.info/software.php).

Exact NEC-values can be calculated for single species. If there is not enough information available for NEC-calculations, PNEC are often calculated by adding a safety factor to the LC_{50} values. Risk assessment can also be performed based on LC_{50}-values (e.g. Brokke 2015, Havforskningsinstituttet 2016). In this project and in Refseth et al. (2016), NEC is calculated for each species. For some species we were not able to calculate NEC, due to high mortality within short time, or when observation was done only once. For species where enough information was not available, we added a safety factor to the LC_{50} to calculate ϱLC_{50}, which is ¼ * LC_{50}. Safety factors are routinely used in ecotoxicological studies if there is not enough information available (dose-response over time).

LC_{50}-values are reported with time unit specifying how long the animals were exposed in the laboratory, e.g. LC_{50} (96 hours). The advantage of NEC is that it is time-independent (i.e. can be calculated regardless of how long the exposure in the laboratory lasts), and therefore NEC is not given a time unit such as LC_{50} (t). NEC can be calculated from acute or chronic toxicity data. The advantage of using NEC over LC_{50} is that sensitivities of species to a chemical can be compared regardless of how long the animals were exposed to the chemical in the laboratory.

To estimate risk at higher level of biological organization (community/ecosystem) rather than estimating risk for single species only, the ecotoxicological metrics such as EC_{50}, LC_{50}- or NEC-values can be assembled into an SSD curve, and a PNEC-value that apply to the given community can be defined (Kooijman 1987) (see chapter 4.2.4).

Endpoint for laboratory studies in the current project is mortality. NEC therefore represents, in this context, the value that will not cause mortality, regardless of how long the animals are exposed. Chronic, sub-lethal effects are not considered in these experiments. In the data collected from the literature, EC_{50} values are also gathered, these data were related to effects on mobility and growth reduction.

To generate ecotoxicological metrics, a toxicokinetic–toxicodynamic (TKTD) model from the framework of the General Unified Threshold Model of Survival (GUTS, Jager et al. 2011) were fitted to the observed mortality patterns over time. The model chosen is called GUTS-RED-SD, and is expressed as follows:

$$h_z = k_k \max(0, C - z)$$

Where, C (mg/L) is the scaled internal concentration and C_d (mg/L) is the environmental concentration. Notice that C has the same dimension as the external concentration. k_d (h^{-1}) corresponds to the dominant rate constant, that is to the slowest compensating process dominating the overall dynamics of toxicity, often referred to as the elimination rate.

Hazard from H_2O_2, h_z (h^{-1}) is taken proportion to the scaled internal concentration once the No Effect Concentration, z (mg/L), is surpassed. The proportionality factor is the killing rate k_k (L/mg/h).

Sometime there are some mortalities which are not explained by the toxicant, these mortalities are induced by some background hazard, h_b (d^{-1}), which is a model parameter. S is the survival probability which is taken proportional to the sum of the two hazard rates.

The TKTD model parameters were estimated from the data via a user-friendly online interface, MOSAICGUTS-FIT, available at http://pbil.univ-lyon1.fr/software/mosaic/guts/, and recommended by the 2018 EFSA scientific option on TKTD effect models for regulatory risk assessment of pesticides for aquatic organisms. It applies Bayesian inference with the MORSE R-package, see Baudrot et al. (2018).

A full description of the GUTS-RED-SD model can be found in Jager & Ashauer (2018).

4.2.4 Species sensitivity distribution curve (SSD curve)

Species generally show different sensitivities to chemicals. SSD is a commonly used tool for environmental risk assessment (ERA). The variation in sensitivity between species can be described by a statistical distribution. A concentration at which x % of species are affected can be derived from the SSD. Usually, an HC_5 (concentration that affects 5 % of species), and a PNEC value for a community is derived, and the values are commonly used in ERA. The PNEC-values used in risk assessment procedures are normally derived from SSD-curves with a safety factor, or from ecotoxicological information from single species (i.e. NEC, or LC_{50} values with a safety factor) or lowest reliable endpoint value is used with an assessment factor

(AF). The size of the AF depends on the uncertainty and the level of extrapolation. PNEC can only be derived from SSD-curves in cases where a relatively large amount of toxicological information on a range of different species is available. Ecotoxicological metrics derived from species from different taxonomic and functional groups can represent sensitivity for a defined ecosystem. The PNEC value represents the sensitivity to whole defined communities rather than sensitivity for a single species (Kooijman 1987). The SSD curve is generated by plotting ecotoxicological values against chemical concentration using a log-normal distribution. PNEC shall be protective for 95% of the species in the ecosystem (Kooijman 1987). Plotting of the SSD assumes that one has point estimates for species from different taxonomic and functional groups. Once a PNEC value is established, this value should be preferred over information on single species when ERA is performed.

Ecotoxicological data available in the literature has been collected and assessed, and the usefulness of these data for risk assessment purposes for H_2O_2 has been evaluated. These data in combination with the ecotoxicological data generated in Refseth et al. (2016), as well as the ecotoxicological data generated in the current projects, provides the raw data for input to the SSD curve (Table 6 - Table 8). Hence, the sensitivity of Norwegian species is included in this curve. The curve has been established based on several assessments and evaluations of available toxicity data. A safety factor can be applied to the SSD, depending on data availability. When there is little data available, a higher safety factor must be applied. The data availability of H_2O_2 is relatively good, hence the safety factor added to the HC_5 value for H_2O_2 is low. Substitute species are often used when there is lack of data for species from different habitats, functional groups etc. Different methodological aspects of SSD for H_2O_2 are provided below and is based on a report in preparation from Nouryon.

4.2.4.1 Intermittent releases of chemicals

It is recognised by the European Chemical Agency (12) that:

"When the environmental exposure will be limited in time, and exposure stops rapidly, populations can tolerate higher concentrations than when it is long lasting. In these cases, short-term LC_{50}-values are used to derive a $PNEC_{water}$, intermittent. Intermittent releases are defined as occurring infrequently, i.e. less than once per month and for no more than 24 hours." Acute ecotoxicity data, i.e. short–term $E(L)C_{50}$ values were used to derive the PNEC for intermittent release and to use this $PNEC_{intermittent}$ in the ERA of H_2O_2.

The environmental exposure to H_2O_2 is infrequent, H_2O_2 is dispersed rapidly after cage or wellboat treatment and it is completely biodegraded. Although it is dispersed and biodegraded, studies have shown that it remains in the environment long enough to exert negative effects (mortality) in some species (Refseth et al. 2016). As previously discussed, sublethal and delayed effects may also occur, but it is beyond the scope of this study to generate PNEC for sub-lethal effect studies.

H_2O_2 is a reactive compound and the mode of toxic action is assumed to be the formation of hydroxyl radicals and subsequent oxidation of biomolecules such as DNA, proteins and membrane lipids. It can easily pass cell membranes and the toxic response will be manifested acutely. This is for example manifested as a low acute to chronic ratio for Daphnids (1 mg/L / 0.63 mg/L) (Reichwaldt et al. 2011, Oplinger &Wagner 2015). To the best of our knowledge there are no data suggesting a specific mode of action for H_2O_2 that would be apparent only after chronic exposure. Hansen et al. (2017), showed that acute exposure (96 h) of a sub-lethal concentration of H_2O_2 (0.75 mg/L) did not cause cellular accumulation of oxidative stress in the marine copepod *Calanus finmarchius*. This is probably because aqueous H_2O_2 exposure

don't cause cellular accumulation, but rather produced acute effects on the copepod surface (carapace).

Acute toxicity data were used for the environmental risk assessment of H_2O_2 in the current project. Acute toxicity data involves ecotoxicological metrics with exposure time in the laboratory from 1 to 96 h. Acute ecotoxicity data, i.e. short-term $E(L)C_{50}$ values, was used to derive a PNEC for intermittent release, and this $PNEC_{intermittent}$ was used in the risk assessment of H_2O_2. Delayed and sub-lethal effects are discussed when risk is assessed in chapter 7.

4.2.4.2 Acute toxicity data

To create the SSD-curve, available ecotoxicity data with relevance have been collected and summarized, focusing on industry reports and studies reported in the open literature that have assessed effects on growth or lethality after 1-96 hours exposure, preferably according to well-recognised guidelines. Most of the presented toxicity data from the public domain are from experiments conducted with a scientific background rather than gearing to registration requirements. Some of these studies are therefore based on non-standardised ecotoxicity tests and the reporting/methods may not perfectly match the Klimisch criteria for data acceptability (Bringman & Kühn1982). The reliability of the studies included in the analysis has been critically reviewed and only studies considered to be of high quality have been included in the derivation of $PNEC_{intermittent}$. The individual studies are presented in Table 6, Table 7 and Table 8.

Reliable ecotoxicity data for 34 species representing 7 phyla are available in articles and reports. The included primary producers are 3 marine diatomes, 5 freshwater green algae and 3 freshwater cyanobacteria. Invertebrate species are represented by 8 marine and 5 freshwater crustaceans, 2 freshwater molluscs and 1 marine rotifer. Fish is represented by 2 freshwater- and 2 marine species. The overall pattern suggested that there was no significant difference in sensitivity between freshwater and marine species. Therefore, species from both environments were included in the SSD-curve. Algal species from the phyla *bacillariophyta* and *cyanobacteria* represents the most sensitive species while marine vertebrates represent the least sensitive trophic level.

4.3 Results and discussion

4.3.1 Ecotoxicological experiments and modelling

The results for measured H_2O_2 concentrations in treatment water are shown in Table 2 for the experiments done by Akvaplan-niva.

Table 2. Nominal and measured H_2O_2-concentrations (mg/L) in treatment water. T0- at start-up, T24- after 24 hours.

Amphipoda			Green sea urchin			Edible crab		
nominal [H2O2]	T0	T24	nominal [H2O2]	T0	T24	nominal [H2O2]	T0	T24
0	0	0	0	0	0	0	0	0
2000	1933	1853	20	20	14	100	110	67
4000	3967	3813	60	65	51	200	206	164
6000	6107	5853	100	100	61	400	474	473
8000	8120	7780	/	/	/	1000	1117	1002
Common whelk juvenile			**Common whelk adult**					
nominal [H2O2]	T0	T24	nominal [H2O2]	T0	T24			
0	0	0	0	0	0			
20	20	19	100	99	95			
50	53	60	400	398	389			
100	93	84	800	799	777			
/	/	/	/	/	/			

The exposure concentration was very stable and close to the targeted nominal concentration. No H_2O_2 was detected in the control tanks. For the analysis of the data we used the mean measured concentration.

4.3.1.1 Amphipods

The first mortalities were observed after 6 hours at the lowest treatment dose of 1 957 mg/L, see Table 3. All individuals died at 24 hours in the highest treatment (7 930 mg/l). Based on the data, a NEC of 1 410 mg/L was estimated. This value is much higher than any NEC that has previously been estimated for e.g. crustaceans, such as *Pandalus borealis* (23mg/l), *Praunus flexuosus* (91 mg/l), or *Praunus elegans* (60 mg/l), or for lumpfish (128 mg/l). The observed survival fraction as function of time is plotted against the predicted survival fraction as function of time in Figure 7. The goodness of fit of model to data can be assessed by looking at the residues. It is very likely that the high resistance of *Gammarus spp.* to H_2O_2 stems from it being an intertidal species. Intertidal species experience large variations in salinity, temperature and drought and are thus in general robust species.

Table 3. Number of surviving amphipods in the control (C) and exposure tanks (C1 – C4). For concentrations see Table 2. T = hours after exposure.

Time (h)	Control	C1	C2	C3	C4
T0	30	30	30	30	30
T1	30	30	30	30	28
T2	30	30	28	26	20
T4	30	30	25	16	12
T6	30	29	22	13	7
T12	30	23	17	8	2
T24	30	20	12	2	0

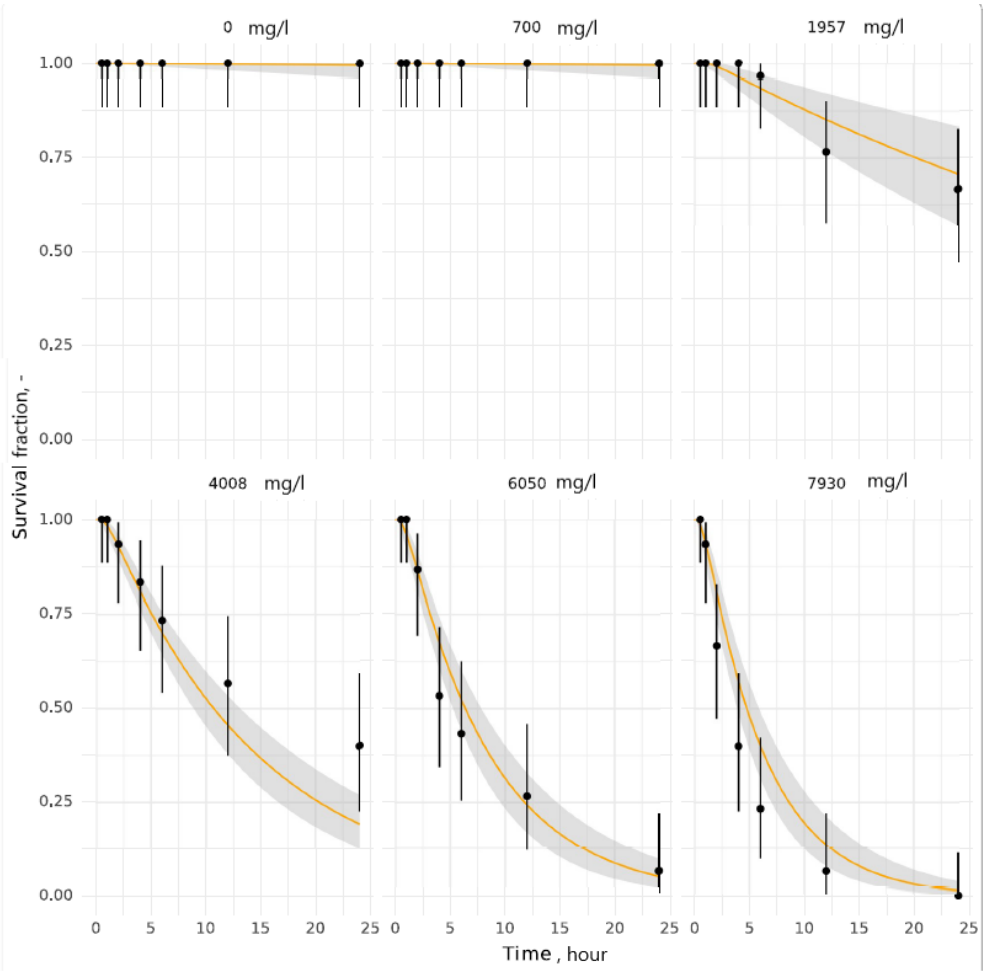

Figure 7. Survival fraction as function of time (hours) for Gammarus spp. Symbols: mean of three replicates, vertical lines: standard deviation. Orange solid line: GUTS-RED-SD prediction. Grey area around the orange solid line: The 95% credible interval for this mean is represented in light grey.

4.3.1.2 Common whelk

As shown in Table 2 the exposure concentration was very stable and close to the targeted nominal concentration. For the analysis of the data the mean measured concentration was used. The first mortalities were observed after 6 hours at the highest treatment concentration of 799 mg/L. All individuals died at 24 hours in the highest treatment (799 mg/L), whereas no individual died throughout the 24 h exposure at the lowest exposure dose (99 mg/L). Based on the data, a NEC of 278 mg/L was estimated. This is higher than any previously estimated NEC-values, except for amphipods, and higher than the juvenile common whelk NEC-value (see Refseth et al. 2016). The experiment on juvenile common whelk in Refseth et al. (2016) revealed a very high sensitivity of the animals to H_2O_2. Unfortunately, survival over time was not possible to estimate since all the animals died short time after exposure to H_2O_2, even in the lowest doses. Hence, since the animals died before the first observation time, LC_{50} value and NEC value could not be calculated (Refseth et al. 2016). The high sensitivity of juvenile common whelk, and the robustness of adult common whelk, illustrates that sensitivity may vary greatly within the same species, depending on the life stage.

The observed survival fraction as function of time for adult common whelk is plotted against the predicted survival fraction as function of time in Figure 8. The goodness of fit of model to data can be assessed by looking at the residues. It is very likely that the high resistance of common whelk adult individuals to H_2O_2 stems from it being a species able to retract into the shell. As such, it is evolved to chemically isolate itself from its environment for extended periods of time. If juveniles are not able to isolate themselves from the chemical by retracting into the shell, it may explain the great variation in sensitivity. Since no LC_{50} or NEC is available for juveniles, we used the value for adults in the SSD curve. When risk is assessed for this species, the sensitivity of juveniles must be considered, to avoid underestimation of risk.

Table 4. Number of surviving adult common whelks in the control (C) and exposure tanks (C1 – C4). For concentrations see Table 2. T = hours after exposure.

Time (h)	Control	C1	C2	C3
T0	20	20	20	20
T1	20	20	20	20
T2	20	20	20	20
T4	20	20	20	20
T6	20	20	20	8
T12	20	20	15	4
T24	20	20	8	0

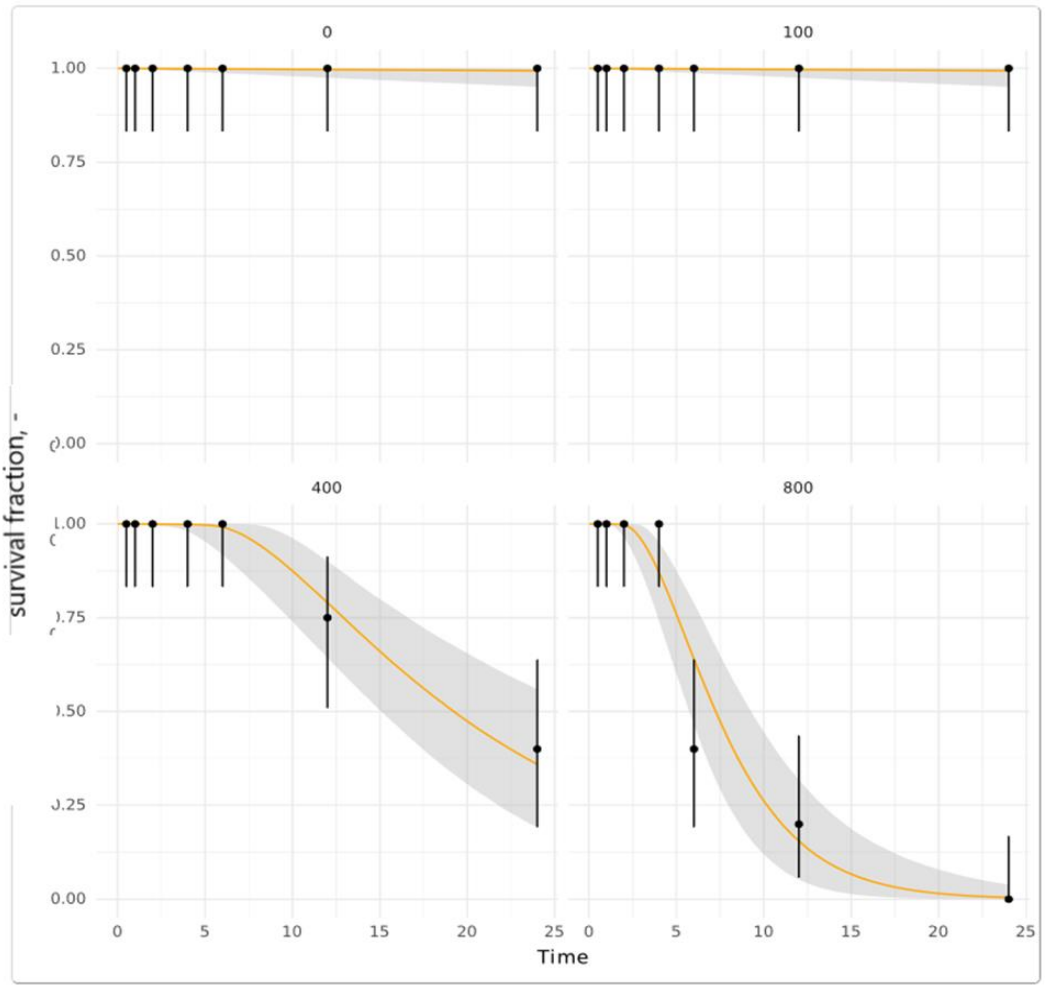

Figure 8. Survival fraction as function of time (hours) for the common whelk Buccinum undatum. Symbols: mean of three replicates, vertical lines: standard deviation. Orange solid line: GUTS-RED-SD prediction. Grey area around the orange solid line: The 95% credible interval for this mean is represented in light grey. Number on top of subfigures indicate the nominal concentration in mg/L.

4.3.1.3 Polychaetes

Capitella sp. and *Ophryotrocha spp.* showed low tolerance to a H_2O_2 treatment dose of 1 800 mg/L, even with a limited exposure time of only 1 h. The results show that 1 h exposures to H_2O_2 at all the tested concentrations had irreversible negative effects on both polychaete species. Both species also showed limited capacity to recover after exposure to all concentrations tested. Most of the polychaetes that were alive after exposure did not survive the recovery period. Therefore, it seems that the damage from H_2O_2 exposure is irreversible in both species and leads to high mortality also at doses that are realistic to find in the environment after delousing.

The mortality after 1 h exposure was considerably lower in *Capitella sp.* than in *Ophryotrocha spp* (Figure 9). However, this difference was reduced at the end of the experiment, as both species experienced a substantial mortality in the recovery period (delayed effects) (for more information see Fang et al. 2018).

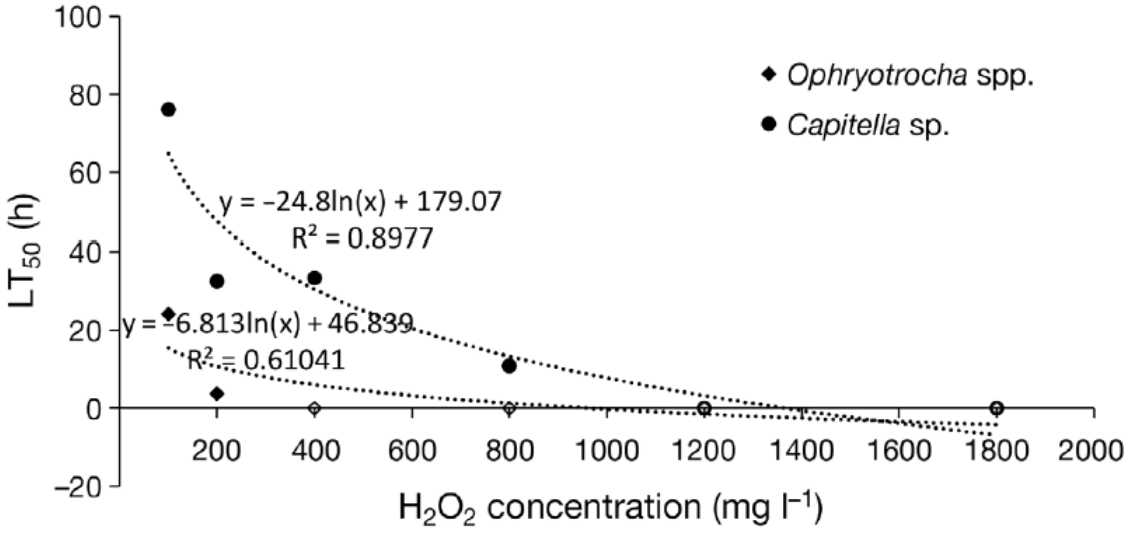

Figure 9. LC_{50} of the polychaete species Capitella sp. and Ophryotrocha spp. at increasing time intervals after exposure to H_2O_2. The equations stem from the results of curve estimation analysis of the relationship between LC_{50} and time (Fang et al. 2018)

4.3.1.4 Sugar kelp

Juvenile *S. latissima* was shown to be highly sensitive to H_2O_2, having an LC_{50} of 80.7 mg/L, which is less than 5% of the dose commonly used at farms and emitted to the environment. A concentration of 85 mg/L caused an immediate 90% reduction in both PMAX (photosynthetic capacity) and α (photosynthetic efficiency). The EC_{50} was found to be 27.8 and 35.4 mg/L for PMAX and α, respectively. Mortality of juvenile *S. latissima* was observed for plants exposed to 85 mg/L (Figure 10). Furthermore, prolonged effects were observed 15 d post-exposure for individuals that survived the 85 mg/L concentration, both in terms of decreased biomass and reduced α and IC (photosynthetic parameter: light requirements for a net photosynthetic rate). The LC_{50}- and EC_{50}-values indicate that *S. latissima* is highly sensitive to H_2O_2 levels that natural local populations could be exposed to from aquaculture emissions, and that *S. latissima* populations in the vicinity of fish farms can be negatively affected by H_2O_2 bath treatments (for more information, see Haugland et al. 2019).

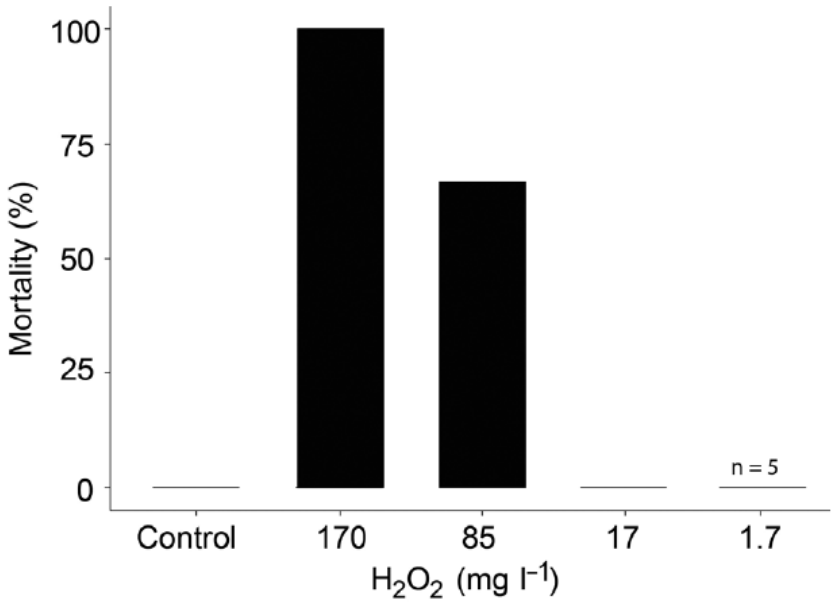

Figure 10. Mortality of juvenile Saccharina latissima plants 15 d after 1 h exposure to 5 different H_2O_2-concentrations, including control (Haugland et al. 2019).

4.3.1.5 Model results

Estimated ecotoxicological metrics for all species exposed, in this and the previous project (Refseth et al. 2016), from the GUTS-RED-SD model are given in Table 5. The NEC varied between 23 mg/l for *P. borealis* and 1 410 mg/l for *Gammarus* sp.

*Table 5. GUTS-RED-SD parameter estimates for all exposed species in this and previous project (Refseth et al. 2016.) 95% credible intervals are in parenthesis. * = 1h exposure followed by 72 h recovery (Fang et al. 2018). ** = 1h exposure followed by 15 d recovery (Haugland et al. 2019).*

	No effect concentration z (mg/l)	**24h exposure LC_{50} (mg/l)**	**Elimination rate k_e (1/h)**	**Killing rate k_k (l/mg/day)**	**Background hazard h_b (1/h)**
Cyclopterus lunpus	128 (88-156)	167	0.12 (0.07-0.18)	0.003 (0.002-0.005)	0.001 ($5.6*10^{-5}$-0.004)
Pandalus borealis	23 (23-38)	37	0.19 (0.12-0.33)	0.003 (0.002-0.004)	0.002
Strongylocentrotus droenbachiensus	Between 10 and 100		-	-	-
Buccinum undatum (juveniles)	Between 10 and 100		-	-	-
Praunus flexosus	98 (54-139)	117	0.08 (0.04-0.34)	0.027 (0.003-0.084)	0.02 (0-01-0.03)
Praunus elegans	60 (0-107)	238	0.06 (0.04-0.43)	0.0005 (0.0001-0.0394)	NA
Gammuras spp.	1410 (940-1700)	2520 (2180-2830)	1.4 (0.755-4.160)	$2.73*10^{-5}$-($2.02*10^{-5}$-$3.61*10^{-5}$)	0,00022 (7.63*10-6-0.0002)
Buccinum undatum (adults)	278 (171-338)	367 (300-414)	0.23 (0,124-0.419)	0.0006 (0.0003-0.0001)	0.0003 ($8.93e^{-6}$-0.002)
Capitella sp.	-	159.3*	-	-	-
*Ophryotrocha spp.**	-	64.3*	-	-	-
*Saccharina latissima***	72.9 ±0.4**	80.7 ±53.5**	-	-	-

4.3.2 Toxicity data collected for SSD generation

This section provides an overview of the different ecotoxicological data which was used to establish the SSD-curve. The data include studies selected from the literature by Nouryon, as well as new data produced in the current project and in the study by Refseth et al. (2016). More results from the data collection and SSD-curve generation will be presented in a separate report (Nouryon in prep.), and part of this text is presented below.

H_2O_2 is a reactive compound and the mode of toxic action is assumed to be the formation of hydroxyl radicals and subsequent oxidation of biomolecules such as DNA, proteins and membrane lipids. H_2O_2 can easily pass cell membranes and the toxic response will be manifested acutely. Consequently, the dose response curves for H_2O_2 are steep, with approximately a two-fold difference between the EC_{50} and the EC_{10}. Modelled $EC_{50/10}$ values are scientifically more robust and accurate compared to NOECs and LOECs which are dependent on the range and distance between the exposure's concentrations used in the assessment. Also, NOECs were only available for 13 species representing five phyla. The $E(L)C_{50}$s, which were available for 35 species were therefore used to derive the $PNEC_{intermittent}$.

The available toxicity data for algae, aquatic invertebrates and fish are discussed in the section below. The data used to generate the SSD curve are presented in Table 6 - Table 8.

4.3.2.1 Toxicity to algae

Effects of H_2O_2 to primary producers have been investigated in numerous freshwater- and marine-algal species from the phyla Chlorophyta, Bacillariophyta and Cyanobacteria (Table 6).

There are two studies with the marine diatom *Skeletonema; Skeletonema sp. and S. costatum.* Both were conducted according to Good Laboratory Practise (GLP) and are considered highly relevant and reliable. The study from Knight et al. (1997) was assigned a Klimish 2 rating because the concentration of H_2O_2 in the test media was only reliably verified at the start of the test (0 hours). In the study from Uzyczak (2019) reliable analytics of H_2O_2 was available, like in the other algal studies. For example, in the study with *Pseudokirchneriella subcapitata* (Chhetri et al. 2017) providing support for the presented EC50s. These three species were all included in the curve.

The toxicity for cyanobacterial species has not been assessed according to a well-recognized guideline. H_2O_2 has been suggested as a potential cyanocidal compound to mitigate cyanobacterial blooms in lakes (Weenink et al. 2015, Matthijs et al. 2016). Matthijs et al. (2016) suggest that five hours of exposure to H_2O_2, at concentrations ranging from 2 mg/L up to 5 mg/L, are appropriate for selective effects on cyanobacteria in freshwater systems, avoiding effects on non-target species, such as eukaryotic algae, invertebrates and fish.

The toxicities to 3 cyanobacteria, 1 diatom and 3 green algae have been reported in the open literature (Florence & Stauber 1986, Clarke 1991). Together this data suggests that some eukaryotic algae are as sensitive as cyanobacteria. For example, the marine diatom *Skeletonema sp*. had the lowest EC50 (0.85 mg/L) that is based on growth rates.

The exposure study by Drábková et al. (2007) suggest that cyanobacteria are more sensitive than diatoms and green algae, but the results are based on a short exposure time (3h), photosynthetic yield and a high irradiation (500 µmol m^{-2} s^{-1}). These results were therefore not included in the analysis. The use of growth rate for estimating toxicity is scientifically preferred (Walzer 1991) and we only included EC50s based on growth rate (ErC50) in the derivation of the $PNEC_{intermittent}$. The yield-based data are included in Table 6 for comparative purposes.

Algae grow exponentially in an algae test and there is therefore in principle no difference when growth inhibition is measured after e.g. 5 hours or 72 hours.

Table 6. Available data on the toxicity of H_2O_2 to algae. [M] Marine species; [F] Freshwater species; [&] calculated from the area under the growth curve or in the study by Drabkova et al. (2007) as inhibition of photosynthetic yield; [&&] calculated from growth rate; n.r. = not reported; n.d. = not determined. The data are included in the SSD curve, however, the list of "additional studies" lists available data that were not included in the SSD.

Guiieline / Test method	Species (phylum)	Endpoint	Exposure (h)	Results [mg/L]			Remarks	Ref.
				NOEC (NEC) / LOEC	EbC50[&]	ErC50[&] [&]		
Paris Commission Guidelines (1990)	*Skeletonema costatum* (*Bacillariophyta*)[M]	Cell density, microscope	72	0.63 / 1.25	1.38	2.62	GLP; analytics; nominal conc.	Knight et al. 1997
Modified ISO 8692	*Pseudokirchneriella subcapitata* (*Chlorophyta*)[F]	Biomass, conc. of chlorophyll	72	/1.78	n.r.	2.9	non GLP; analytics; nominal conc.	Chetri et al. 2017
Modified OECD 201	*Chlorella vulgaris (Chlorophyta)[F]*	Cell density	72	0.1 / 0.25	2.5	4.3	non GLP; no analytics	Walzer 1991
Other	*Nitzschia closterium* (*Bacillariophyta*)[M]	Cell density, haemocytometer	72	-- / 0.68	n.r.	0.85	non GLP; analytics ; nominal conc.	Florence and Stauber 1986
Other (microtiter plates)	*Anabaena variabilis (Cyanobacteria)[F]*	Biomass, measured as absorbance at 630 nm	13- 140	n.r.	n.r.	~5	non GLP; no analytics	Clarke 1991
	Anabaena A4 (Cyanobacteria)[F]		13-140	n.r.	n.r.	~1.6		
	Synechococcus leopoliensis (Cyanobacteria)[F]		13-140	n.r.	n.r.	~10		
	Chlamydo-monas eugametos (*Chlorophyta*)[F]		45-240	n.r.	n.r.	~20		
	Chlorella emersonii (*Chlorophyta*)[F]		45-240	n.r.	n.r.	~17		
	Scenedesmus quadricauda (*Chlorophyta*)[F]		45 -240	n.r.	n.r.	~28		
Based on Andersen et al. 2013	*Saccharina latissimi (Phaeophyceae)[M]*	Mortality, photosynthetic capacity and efficiency	1			*80.7 ± 53.5*	Nominal concentration 1h exposure followed by 15 d recovery	Haugland et al. 2019
Based on ISO 10253	*Skeletonema sp* (*Bacillariophyta*)[M]	Growth	72	0.41/0.74		0.59	GLP, analytics, geometric mean measured conc.	Uzyczak 2019
Additional studies:								
Other (microtiter plates)	*P. subcapitata* (*Chlorophyta*)[F]	Inhibition photosynthetic yield: test performed @ irradiance 500, 40 and 0 µmol m^{-2} s^{-1}, respectively	*3*	n.r.	4.15, 6.09, 21.26	n.d.	non GLP; analytics; nominal conc.	Drábková et al. 2007
	Navicula seminulum (*Bacillariophyta*)[F]		*3*	n.r.	15.78, 12.19, 71.26	n.d.		
	Microcystis aeruginosa (Cyanobacteria)[F]		*3*	n.r.	0.27, 0.45, 6.63	n.d.		
Based on ISO 10253	*S. costatum* (*Bacillariophyta*)[M]	Reduced activity and concentration of active chlorofyll a	24.5	(2.78)	n.r.	n.r.	non GLP; analytics; nominal conc.	Smit et al. 2008
	Dunaliella tertiolecta (*Chlorophyta*)[M]		24	(2.14)	n.r.	n.r.		

4.3.2.2 Toxicity to aquatic invertebrates

Effects of H_2O_2 on aquatic invertebrates have been investigated in 11 marine and 7 freshwater invertebrates (Table 7), together representing 3 different phyla (*Arthropoda*, subpylum *crustacea*; *Rotifera* and *Mollusca*). The subphylum *crustacea* is diverse and important in the marine environment and this subphylum was represented by three classes (Table 7).

The test on the marine crustacean *Calanus finmarchicus* in Hansen et al. (2017) was performed following ISO test, and the experiments performed on *Pandalus borealis* and *Gammarus sp.* were conducted based on OECD 23 standard (Refseth et al. 2016). Test on *Praunus flexuosus* and *Praunus elegans* were conducted using other non-standardized methodologies (Refseth et al. 2016). Comparing mortality studies among the marine crustaceans, the adult *C. finmarchicus* and *P. borealis* were the most sensitive, with 1 h LC_{50} of 35 mg/L and 24 h LC_{50} of 37 mg/L, respectively. *Gammarus sp.* was the most robust with an LC_{50} of 2 520 after 24 h exposure.

The study with the freshwater species *Daphnia pulex* was conducted according to USA EPA guideline, included analytics, but was not conducted according to GLP (Shurtleff 1989). The range of test concentrations was sub-optimal since there was no immobility in the lowest test concentration group and 100 % at 24 h in the remaining 5 test concentrations, but the study was considered reliable.

Additional studies, conducted according to recognised guidelines, provide validating evidence for a crustacean EC50 of a few mg/L. For example, the EC_{50} of the marine copepod *C. finmarchius* and two water fleas ranged between 2 and 5.6 mg/L. The concentration of H_2O_2 was not analytically confirmed, however the test design was adjusted to prevent degradation; i.e. no aeration and minimised light exposure was used. The freshwater mollusc *Potamopyrgus antipodarum* was somewhat less sensitive than the crustaceans and the study with *Physa sp.* and *Gammarus sp*. also suggest that molluscs generally are less sensitive than crustaceans (Kay et al. 1982).

Overall, the data suggest that, compared to algal species, invertebrates are slightly less sensitive to H_2O_2 exposure. The freshwater crustaceans *D. pulex* and *Moina sp.* and the marine rotifer *Brachionu plicatilis* had the lowest EC50s, around 2 mg/L.

Table 7. Available information about the toxicity of H_2O_2 to aquatic invertebrates. [M] Marine species; [F] Freshwater species. The data are included in the SSD curve.

Guideline / Test method	Species (Subphylum/, subclass)	Endpoint	Exposure		Results [mg/L]		Remarks	Ref.
			design	duration	EC_{50}	NOEC (NEC)		
US EPA Guidelines, 40 CFR Parts 796, 797, 798 (1985, 1987)	*Daphnia pulex (Crustacea,, Diplostraca)*[F]	Mortality	Semi-static	48 h	2.4	1	non GLP; analytics; measured conc.	Shurtleff 1989
Based on standard test on Acartia tonsa (ISO)	*Calanus finmarchius (Crustacea, Copepoda)*[M]	Mortality	Static	48, 72 and 96 h	3.9, 3.8 and 2.5	0.75 (96h)	non GLP; no analytics	Hansen et al. 2017
Other	*Calanus finmarchius adult (Crustacea, Copepoda)*[M]	Mortality	Static	1 h	35	9	non GLP; no analytics	In Refseth et al. 2016, data from Escobar Lux
Other	*Calanus finmarchius (copepoditt stage V) (Crustacea, Copepoda)*[M]	Mortality	Static	1 h	173	43	Non GLP; no analytics	
Other	*Potamopyrgus antipodarum (Mollusca, Caenogastropoda)* [F]	Mortality, response to tactile stimuli	Static	24 and 48 h	37.5 and 11.0	n.r.	non GLP; no analytics	Oplinger & Wanger 2015
Other, similar to OECD 202	*Daphnia carinata (Crustacea,Phyllopoda)*[F]	Mortality	Static	48 h	5.6	3	non GLP; no analytics	
Other, similar to OECD 202	*Moina sp. (Crustacea, Branchiopoda)*[F]	Mortality	Static	48 h	2	1.5	non GLP; no analytics	Reichwaldt et al. 2011
Artemia reference center test (Vanhaecke and Persoone 1984)	*Artemia salina (Crustacea, Sarostraca)*[M]	Mortality	Static	72 and 96 h	188 and 168	(133)	non GLP; analytics; nominal conc.	Smit et al. 2008
Method described in (Schipper et al 1999)	*Brachionus plicatilis (Rotifera, monogononta)*[M]	Mortality	Static	26 h	2.4	(1.87)	non GLP; analytics; nominal conc.	Smit et al. 2008
Modified standard test: (Schipper et al 1999)	*Corophium volutator (Crustacea, Eumalacostraca)*[M]	Mobility / acute	Static	96 h	46	n.r.	non GLP; analytics; nominal conc.	Smit et al. 2008
OECD 23 Other	*Pandalus borealis (Crustacea, Eumalacostraca)*[M]	Mortality	Static	24 h	37	(23)	non GLP; analytics; measured conc	Refseth et al. 2016
OECD 23	*Gammarus sp. (Crustacea, Eumalacostraca)*[F]	Mortality	Semi-static	96 h	4.4	n.r.	non GLP; test conc. not given	Kay et al. 1982
Other	*Physa sp. (Mollusca)*[F]	Mortality	Semi-static	96 h	17.7	n.r.	non GLP; test conc. not given	Kay et al. 1982
DIN 384102 L 11	*Daphnia magna (Crustacea, Diplostraca)*[F]	Immobility	Static	24 h	7.7	3.8	non GLP; test conc. not given	Bringmann & Kühn 1982
ISO 14669 (1999)	*Tisbe battagliai*[M] *(Crustacea, Copepoda)*	Mortality	Semi-static 24h renewal	72h	10.48 (24h) 8.90 (48h) 2.53 (72h)	<0.28 (72h)	GLP, analytics, geometric mean measured	Uzyczak 2019
Other	*Praunus flexuosus*[M] *(Crustacea, eumalacostraca)*	Mortality	Static	24h	117	98	Nominal concentration	Brokke 2015

Other	*Palaemon elegans*[M] *(Crustacean eumalacostraca)*	Mortality	Static	24h	238	60	Nominal concentration	Brokke 2015
OECD 23	*Gammarus sp*[M] *(Crustacean)*	Mortality	Static	24h	2520 (2180-2830)	1410 (940-1700)	Analytics, measured concentration	APN report in prep
OECD 23	*Buccinum undatum*[M] *(Mollusca, Caenogastropoda)*	Mortality	Static	24h	367 (300 - 414)	278 (171 - 338)	Analytics, measured concentration	APN report in prep
Other	*Capitella sp*[M] *(Annelida)*	Mortality	Static	1h	159.3		Analytics, measured concentration, 1h exposure followed by 72 h recovery	Fang et al. 2018
Other	*Ophryotrocha spp*[M] *(Annelida)*	Mortality	Static	1h	64.3		Analytics, measured concentration, 1h exposure followed by 72 h recovery	Fang et al. 2018

4.3.2.3 Toxicity to fish

The acute toxicity of H_2O_2 to fathead minnow (*Pimephales promelas*) has been assessed according to US EPA TSCA Test Guidelines, 40 CFR Parts 796, 797, 798 (1985, 1987 (revision), but as it is an older study it was not conducted according to GLP (Shurtleff 1989). Acute toxicity data for 1 freshwater and 2 marine fish were also available in the open literature. Together this data provides further support for that fish represent the least sensitive trophic level (Table 8).

Table 8. Available information about the toxicity of H_2O_2 to fish. [M] Marine species; [F] Freshwater species. Data rae included in the SSD curve.

Guideline / Test method	Species	Endpoint	Exposure		Results [mg/L]		Remarks	Ref.
			design	duration	LC_{50}	NOEC (NEC)		
US EPA	*Pimephales promelas*[F]	Mortality	Semi-static	96 h	16.4	4.3	non GLP; analytics; measured conc.	Shurtleff 1989
Other	*Ictalurus puctatus* [F]	Mortality	Semi-static	96 h	37.4		non GLP; nominal conc.	Kay et al. 1982
Other	*Cyclopterus lumpus*[M]	Mortality	Static	24 h	167	(128)	non GLP; measured conc.	Refseth et al. 2016
Other	*Gadus morhua* [M]	Mortality	Static	24 h	342	(147)	non GLP; nominal conc.	Refseth et al. 2016

4.3.2.4 Statistical derivation of $PNEC_{intermittent}$

If a large dataset of ecotoxicity data for different taxonomic groups is available, statistical extrapolation methods may be used to derive a PNEC and the assessment factor can be reduced if the coverage of species is representative of the exposed ecosystem (ECHA 2008).

It is recommended that a PNEC derived by statistical extrapolation is based on effect concentrations from at least 10 species (preferably more than 15) covering at least eight taxonomic groups. Deviations from these recommendations can be made, on a case-by-case basis, through consideration of sensitive endpoints, sensitive species, mode of toxic action

and/or knowledge from structure-activity considerations (ECHA 2008). An assessment factor between 1 and 5 are recommended to derive a PNEC from a species sensitivity distribution (SSD) (ECHA 2008).

Here we generated and analysed the SSD using the ETX program (version 2.2) (Van Vlaardingen 2004).

Reliable ecotoxicity data for 34 species representing 7 phyla were available to derive the SSD (Table 6 - Table 8). The distribution of effect concentrations was log-normally distributed, and the pattern suggested that there was no significant difference in sensitivity between freshwater and marine species.

4.3.2.5 SSD curve for H_2O_2

Based on the data described above, the SSD curve was generated and analysed using the ETX program (version 2.2). The curve includes the toxicity data for the Norwegian species tested.

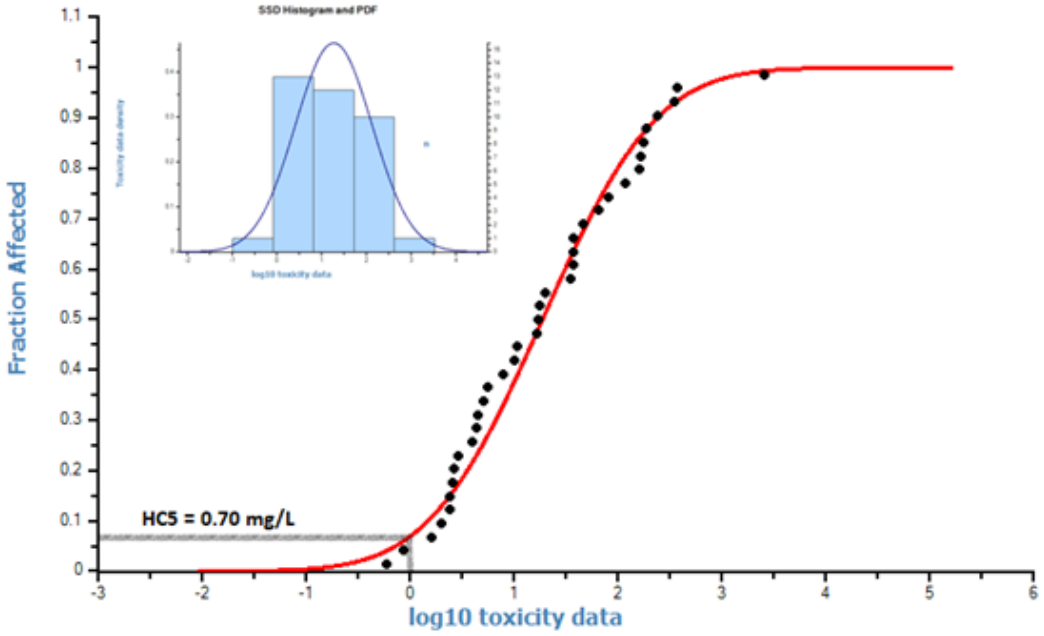

Figure 11. ***Species sensitivity distribution (SSD) of H_2O_2 based on acute toxicity data, E(L)C_{50}s, derived from 34 species representing seven different phyla****. There is no apparent difference in sensitivity between fresh-water and marine species. Algal species from the phyla cyanobacteria and bacillariophyta represent the most sensitive species while marine vertebrates represent the least sensitive trophic level (figure created by Nouryon)*

The hazardous concentrations (HC_5, i.e. derived at a 95% protection limit) were estimated to be 0.70 mg/L. Algal species from the phyla *cyanobacteria* and *bacillariophyta* represented the most sensitive species while marine vertebrates generally represent the least sensitive trophic level.

An assessment factor between 1 and 5 are recommended to derive a PNEC from a species sensitivity distribution (SSD) (ECHA 2008). The assessment factor should justify extrapolation from EC_{50}s to NOECs, from laboratory to field and from 34 species to all species in the exposed

marine environment. H_2O_2 is a reactive compound and the dose response curves were generally steep, with approximately a two-fold difference between EC_{50}s and EC_{10}s. The distribution of L(E)C_{50}s values was log-normally distributed suggesting a good representation of species and phyla and provides further support for a similar mode of toxicity across the different species and taxa. Together this suggests that an assessment factor of 5 provides an appropriate assessment factor.

The derived $PNEC_{intermittent}$ was determined to 0.14 mg/L and it is lower than all but one of the NOECs. The NOEC for the freshwater green algae *C. vulgaris* was 0.1 mg/L but its LOEC (0.25 mg/L) are higher than the $PNEC_{intermittent}$ suggesting that a concentration of 0.14 mg/L H_2O_2 would not affect *C. vulgaris.*

The lowest yield based EC_{50} (0.27 mg/L) was from a study with the cyanobacteria *M. aeruginosa* and was based on inhibition of photosynthetic yield (which often is a more sensitive endpoint that the specific growth rate) and at the highest irradiance tested. No inhibition of the photosynthetic yield of *M. aeruginosa* was measured at 0.14 mg/L H_2O_2 at the highest irradiance (Drábková et al. 2007) suggesting that a $PNEC_{intermittent}$ of 0.14 mg/L would be protective also of cyanobacterial species.

5 Input from the industry

The project aims to evaluate risk from discharges of H_2O_2 after treatment against sea lice. Modelling should therefore be based on relevant discharge scenarios. Delousing of salmon in Norwegian aquaculture is done either in wellboats or directly in the cages with the use of tarpaulin. In the current project, representatives from the industry are partners to ensure use of relevant scenarios for release of treatment water.

The salmon is treated with H_2O_2 in the recommended concentrations for up to 20 minutes until the lice fall off and die. While it is true that H_2O_2 decomposes relatively quickly, the use of higher concentrations of H_2O_2 can have negative effects on the salmon. The concentration must therefore always be within the specifications during treatment (Table 9).

The toxicity increases with increasing concentration, temperature and exposure time. Salmon can survive exposures to a concentration of 1 500 mg/L at temperatures up to 18°C, at exposure times less than 30 minutes.

Table 9. Specifications for treatment varies with temperature.

Water temperature	Concentration of H_2O_2
< 4 °C	2.1 g/L ± 0.2 g/L
>4°C to 8°C	1.8 g/L ± 0.2 g/L
>8°C to 14°C	1.5 g/L ± 0.2 g/L

To achieve optimal efficiency and safe treatment, the calculation of the treatment volume must be accurate, and the dosage and administration instructions must be followed.

The producer of H_2O_2 recommends that treatment should be avoided when seawater contains large amounts of organic material or when the nets are fouled, as this can reduce the effect of the treatment.

During treatment, the concentration of H_2O_2 must be monitored and maintained at the correct level, as shown in Table 9. Duration of the treatment is 20 minutes from the end of dosing.

The scenarios for release of H_2O_2 that have been modelled and used for risk assessment have been selected in cooperation with Aqua Pharma and Sølvtrans, representing expertise on the use of H_2O_2 in tarpaulins and in wellboats.

5.1 Delousing in tarpaulin

Use of bath treatment in cages with tarpaulin, causes limited stress for the fish, as it receives treatment in its own habitat, without being moved by pumping, crowding and with minimum action. When using H_2O_2 it is essential that the treatment takes place under controlled conditions. The water quality, salinity, turbidity, oxygen, CO_2, temperature, dosing and exposure time are parameters that must be under control, to maintain fish welfare during treatment.

The site/fish farm shall be prepared for treatment, i.e. equipped with test dosing equipment and equipment for analysis of H_2O_2 and oxygen. Dosing and routines for analysis are tested and

personnel trained. Oxygenation starts before the tarpaulin is mounted. The oxygen concentration is monitored at several depths in the cage.

Delousing in tarpaulin is weather dependent. Maximum current on the site cannot be higher than 35 cm/sec. Water transparency must be minimum 6 m at start. The process requires dedicated service vessels with sufficient capacity and equipment.

There are several different cages that are used in Norwegian aquaculture, and different tarpaulins.

"Flat tarpaulin" is well suited for big volumes (14-15 000 m^3) and provides a large effective volume for the fish. This type works best when set outside the bottom ring and float collar.

"China hat" is best suited for smaller biomasses and small volumes. The tarpaulin is set inside the net.

"Muffin" is a shaped cylinder. It has stabile volume when used correct. The circumference of the tarpaulin is 3-5 % larger than the cage ring, and it has a large effective volume for the fish. This tarpaulin can withstand more than 50 % higher current than other tarpaulins.

The cage net bottom is raised to form a doughnut shape. Required equipment, like oxygen meter, oxygen supply, stirring and titration hose, is placed in the cage. Determination of water makes a basis for how to set the tarpaulin, which should be done against the water current. Once the tarpaulin is closed, H_2O_2 should be added as fast as possible. When the treatment period is over the tarpaulin is removed as fast as possible, releasing treatment water at once/at the same time.

5.2 Delousing in wellboat

Most wellboats can perform delousing, and Sølvtrans has provided input on how delousing in wellboat is performed.

Regulations requires that the wellboat shall be adapted for treatment with H_2O_2. The boat must have proven equipment, including dosing pump, hose system and round pumping system, for even distribution of H_2O_2 in tanks and oxygen level measurement. Dosing and analytical routines must be tested, and the personnel trained.

Salmon is pumped from the cage into the wellboat. When the fish are on board, dosing of H_2O_2 starts. Oxygen content in treatment water is measured during the operation. H_2O_2 is dosed into the circulating flow by circulation pumps to maintain concentration in accordance with the dosing recommendations (Table 9). The concentration of H_2O_2 is followed carefully during dosing to maintain target concentration. Amount H_2O_2 depends on water temperature, amount of fish and water in the well. It takes approximately 15-20 minutes to reach the correct therapeutic doze in the well.

After the treatment, flushing starts by pumping seawater into the well, and flushing treatment water out. Because of the continuous addition of clean seawater, the water flushed out will be continuously diluted. After approximately 15-20 minutes all the H_2O_2 is flushed out, and the well consist of only salmon and clean seawater.

Most wellboats will have more than one chamber. Depending on the biomass in the cage that shall be deloused, all chambers will be filled with salmon. Dosing of chamber one is completed before starting dosing of chamber two and so on. Flushing of treatment water will appear as separate discharges for the different chambers.

The wellboat will normally discharge the water in a distance from the salmon site. Treatment water is flushed out while the boat is going at a low speed at approximately 7 knot.

New regulations from 2017 restricts discharge of treatment water. Treatment water cannot be discharged to sea closer than 500 meters from shrimp fields or spawning grounds, i.e. the fields that at any time is displayed in the Directorate of Fisheries' web-based map tools. The regulations further require that when discharge of treatment water takes place elsewhere than at the aquaculture site, the water must be emptied while the vessel is in motion. (Forskrift om transport av akvakulturdyr, § 22a.).

5.3 Input to modelling

5.3.1 Location

Delousing with H_2O_2 is used in all regions of Norway. However, in 2015 the use of H_2O_2 was most frequent in West- and Mid-Norway (Remen & Sæther 2018). In 2017 the situation changed, as the most frequent use then was recorded in Northern Norway. As shown in Refseth et al. (2016), environmental impacts from H_2O_2 depend on concentration and exposure time. Dilution of H_2O_2 is mainly driven by currents, and impact of release from a cage might differ strongly between different geographical sites.

Model sites from 4 different geographic areas in Norway were chosen. The sites are chosen to represent difference between fjords and coastal waters. The sites furthermore have different depths, varying between 50 and 120 m.

5.3.2 Number of cages

A mapping of "Use of therapeutics against sea lice in Norway in 2012 – 2017" (Remen & Sæther 2018) showed that fish feed with delousing therapeutics usually were administered to all cages at a site at the same time. However, for bath treatments there has been a development from delousing the whole site (common in 2012) to delousing one cage at the time (common in 2017). This trend was particularly relevant for the use of H_2O_2. Delousing single cages were more likely in western and middle part of Norway than in northern part of Norway (Remen & Sæther 2018). Furthermore, most of the delousing processes with H_2O_2 in wellboats were done for single cages, and not the total site.

We have chosen to model releases from one single cage, as well as from four cages (released from one cage at a time). This is done for one of the sites, to assess potential differences in environmental impact.

5.3.3 Volume tarpaulin

Fish farmers in Norway use different cage sizes. Most common are cages with a circumference of 90 m, 120 m and 160 m. According to regulations, the density of fish cannot exceed 25 kg/m^3. This means that at least 97.5 % of the volume in the cage is water. When delousing in tarpaulin, the cage is compressed. The amount of H_2O_2 used for delousing will differ between different cages, tarpaulin type, and the filling level of the tarpaulin.

In this project, there is information from Aqua Pharma on specific volumes they deloused in 2017. The most common cage size deloused had a circumference of 120 m. This information is used as input to the modelling in the project.

In the modelling the maximum volumes observed during delousing (14 900 m^3 for 120 m and 21 000 m^3 for 160 m) have been used.

5.3.4 Volume wellboat

When delousing in wellboat, volume depends on the biomass of the fish and size of the boat.

Modern large wellboats are equipped with filter for de-lousing-system. We have chosen to use a large wellboat (3 000 m^3) with 3 wells when modelling releases from wellboat.

Recommended density of fish in the well when de-lousing is 100 kg/m^3. Data from our case study shows that density varied from 85 kg/m^3 to 113 kg/m^3. 100 kg/m^3 was chosen for the model scenario.

As described above, it is common to de-louse single cages at a time when using wellboats (Remen & Sæther 2018). Furthermore, for the larger the fish, it is more common to use H_2O_2, as shown in Figure 12 (blue color shows use of H_2O_2).

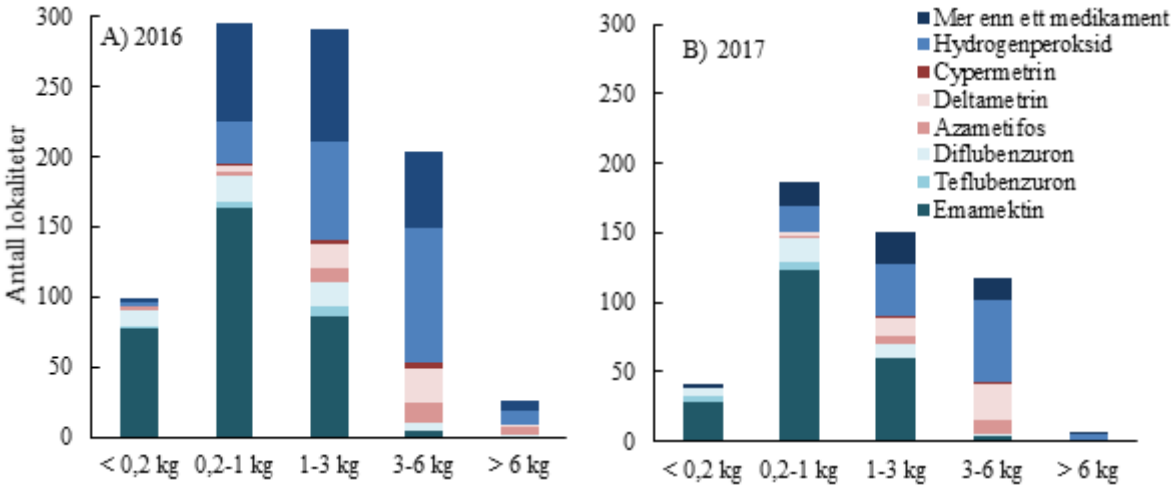

Figure 12. Number of sites that have requisitioned de-lousing agents in A) 2016 and B) 2017, by fish size. One site may be represented several times (Remen & Sæther 2018). Y-axis shows number of sites.

According to regulations, the number of fishes in one cage may not exceed 200 000. If average fish size is 3 kg, this gives a total amount of 600 tons in the cage. With capacity of 100 kg/m^3, a wellboat of 3000 m^3 need two treatments to delouse one cage. If average size is 4.5 kg, this requires three treatments to delouse one cage of 200 000 fish. In our case study two cages with approximately 110 000 fish per cage were treated. The average fish size was 6 kg, giving a total biomass per cage of 660 tons. The wellboat had to perform four consecutive treatments to de-louse the two cages.

From experience, one wellboat can manage 2 – 3 rounds per day, depending on the distance between the site and the drop zone among others. As input to modelling, we have used two treatments per cage, and two rounds per day.

Total volume in the wellboat chosen is 3 000 m^3. However, fish will displace water; 1 kg fish displace approximately 1 L of water. Total treatment volume in each of the 3 well is 950 m^3.

6 Modelling the release of H_2O_2 from fish cages and wellboats

6.1 Introduction

H_2O_2 is denser that water, and the density of the mixture of seawater and H_2O_2 used for delousing is about 1-2 per mill larger than the surrounding seawater. In a weakly stratified water mass, this leads to a rapid sinking after release. The sinking will occur within a few minutes after release and has an important impact on the spreading of H_2O_2 in the environment.

6.2 The model

We have used the Finite Volume Community Ocean Model, FVCOM (Chen C. H., 2003), to model the dispersion of H_2O_2 in the environment. Due to its unstructured grid, FVCOM is particularly suited to model oceanic flows in regions with fractured coastlines and archipelagos. FVCOM is used all over the world for aquaculture related challenges (Foremen et al. 2015, Aleynik et al. 2016, Adams et al. 2016).

The equations used to estimate transport from one point on the grid to another are called "advection schemes". We have used the MPDATA (Smolarkiewicz 1984) scheme for vertical transport. This was already implemented in FVCOM, but we have modified it to account for precipitation and evaporation (Appendix 1). Due to the approximations made in advection schemes, some numerical diffusion – model induced, unphysical transport – is expected. We have updated FVCOM with a Total Variation Diminishing (TVD) scheme (Harten 1983) for FVCOM, which decreases this artificial transport compared to the original scheme (Appendix 1).

6.3 Model domain

FVCOM is setup for the coast of Nord-Trøndelag, which is located in mid-Norway. The model domain and bottom topography is shown in Figure 13. NorKyst800 (Albretsen 2011), a ROMS based model of the Norwegian coast with 800 m resolution, forces the model at the open boundary. The atmospheric forcing comes from WRF model simulations by the Institute of Marine Research (Heikkilä et al. 2011, Myksvoll et al. 2012). The model includes 2 524 rivers, and river runoff data is obtained from Norges Vassdrags og Energidirektorat (NVE). The grid consists of 492 212 nodes, 946 770 cells and there is 35 layers in the vertical. Horizontal resolution varies from 30 m in narrow passages near the coast, to about 1 km near the open boundary. The mesh is shown in Figure 14. Model results are stored every hour throughout the year 2014.

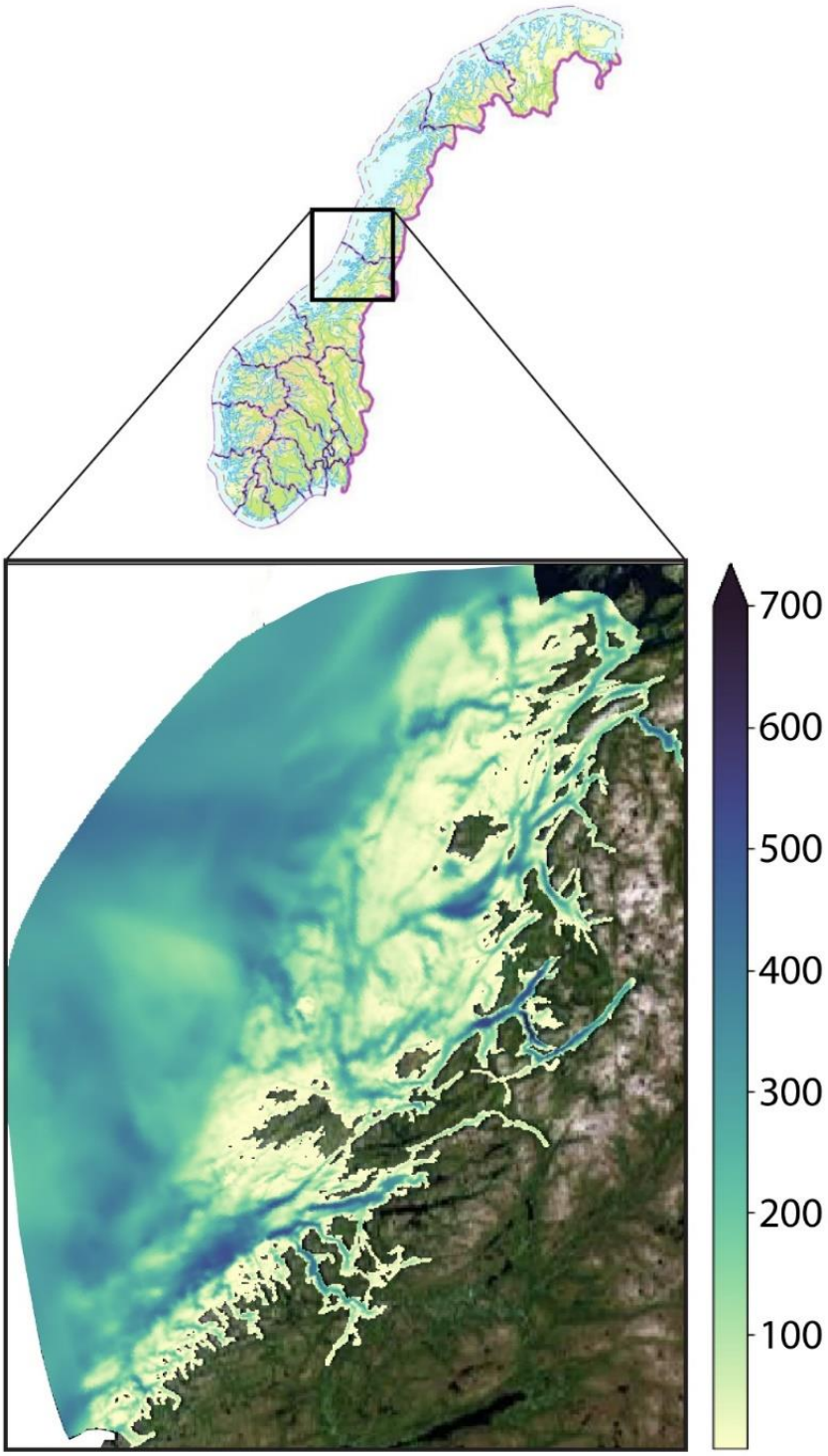

Figure 13. The model domain covering northern Trøndelag in mid-Norway. The bottom topography is shown in the lower panel.

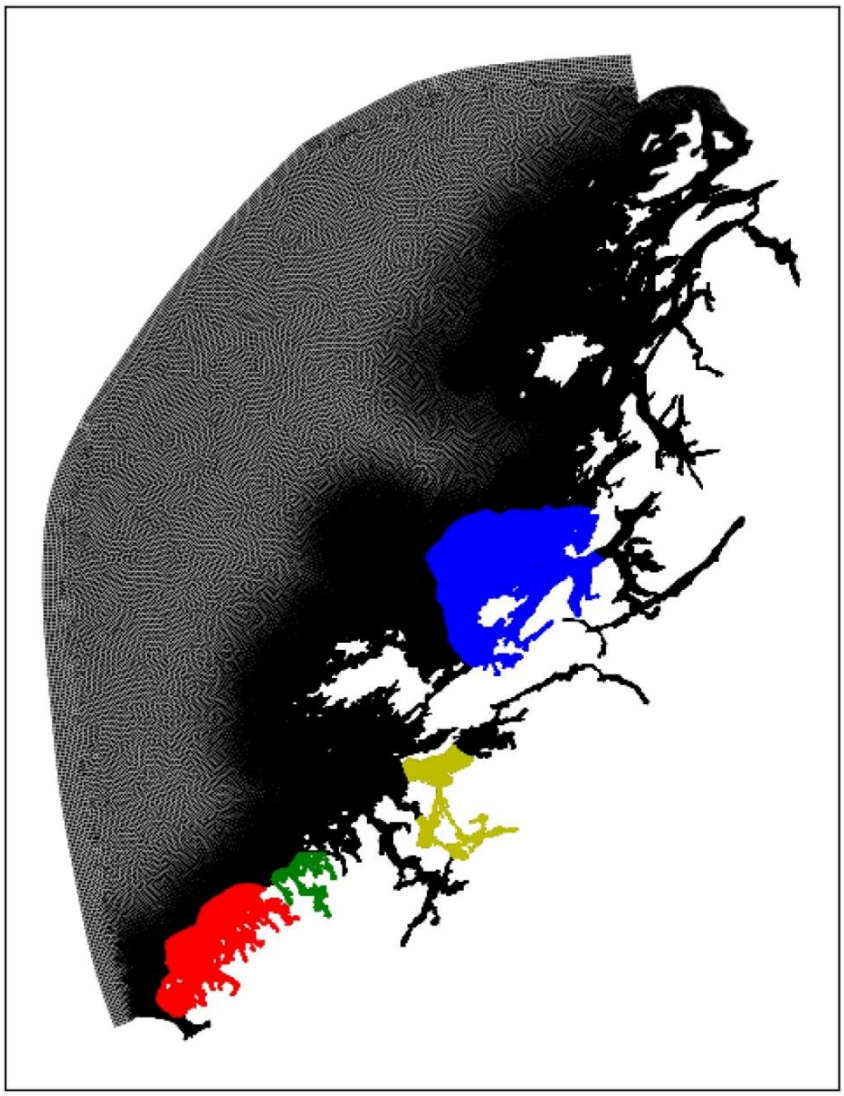

Figure 14. The model mesh. The large mesh is shown in black while the mesh of the four sub-regions is shown in red (Indre Skjervøy), green (Austvika), light green (Kjelneset) and blue (Jakobsteinsvika).

6.3.1 Nested domains

Simulating the sinking and spreading of H_2O_2 from aquaculture locations require very high resolution, in order to model the sinking and dispersion that occurs minutes after release. Therefore, we have nested four small high-resolution models into the main model domain. The nested domains are shown as colored areas in Figure 14.

6.3.1.1 Indre Skjervøy

Indre Skjervøy is an exposed site far out towards the open ocean. The domain is shown in red in Figure 14, and the high-resolution mesh and topography is shown in Figure 15. The mesh resolution is about 10 m at the release point, shown in red. Deep trenches in between shallow areas near the coast characterize the topography in the domain. The depth at the release point is 120 m.

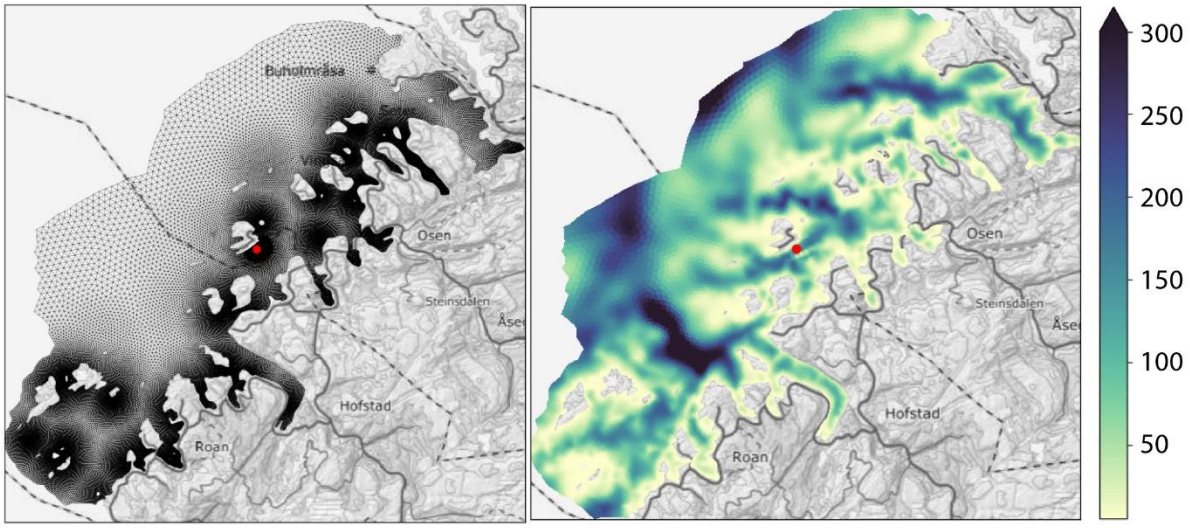

Figure 15. The mesh (left) and bottom topography (right) for Indre Skjervøy. The red point shows the location of the release of H_2O_2.

6.3.1.2 Austvika

Austvika is located within Jøssundfjorden. The fjord is about 1 km wide at the entrance. The depth at the release point is about 100 m, and depth inside the fjord is up to 250 m. At the entrance there is a sill of about 50 m depth. The domain is shown in green in Figure 14, and the high-resolution mesh and topography is shown in Figure 16. The mesh resolution is about 10 m at the release point, shown in red.

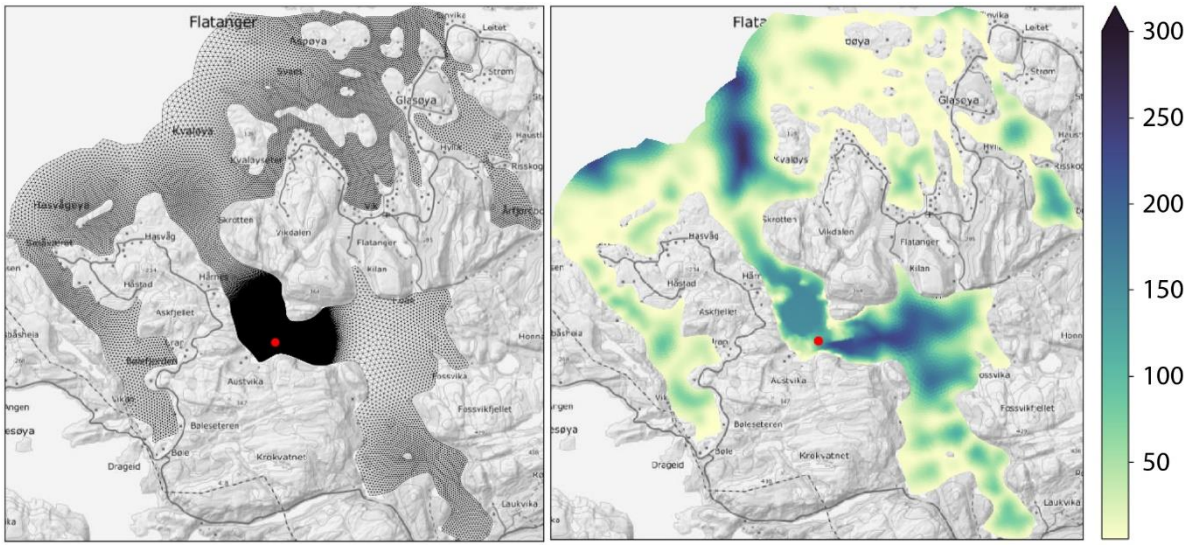

Figure 16. The mesh (left) and bottom topography (right) for Austvika. The red point shows the location of the release of H_2O_2.

6.3.1.3 Kjelneset

Kjelneset is located in Gyltfjorden. The fjord is about 1-2 km wide. The domain is shown in light green in Figure 14, and the high-resolution mesh and topography is shown in Figure 17. The depth at the release point is 50 m, and the depth in the fjord is up to 200 m. To the north the fjord is open towards Folda, which is a wider and deeper fjord. To the south there is

narrow passages connecting Gyltfjorden to the fjords further inland. There is no sill in Gyltfjorden.

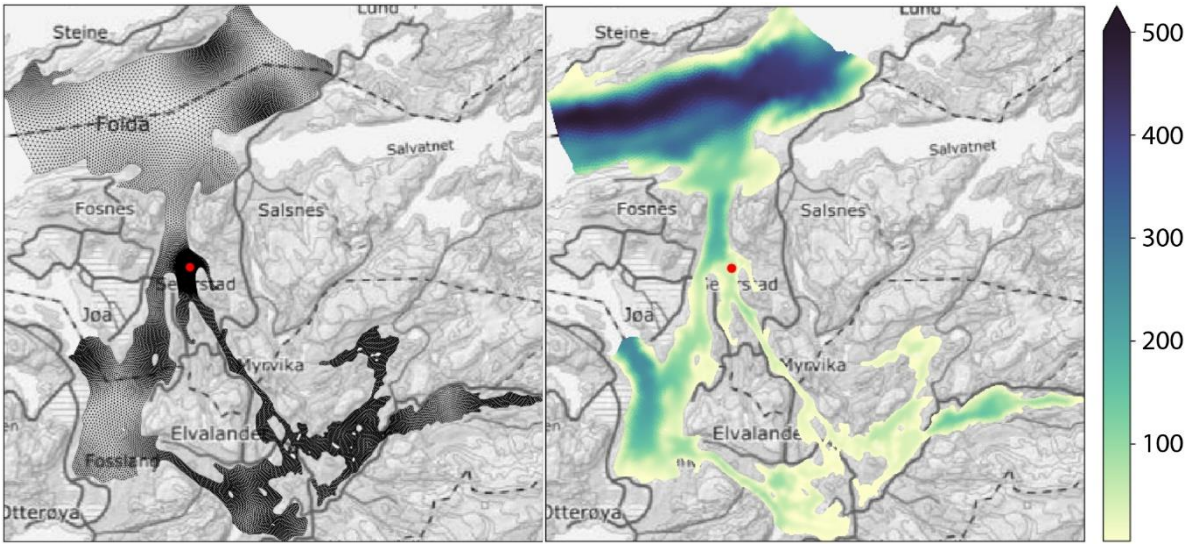

Figure 17. The mesh (left) and bottom topography (right) for Kjelneset. The red point shows the location of the release of H_2O_2.

6.3.1.4 Jakobsteinsvika

Jakobsteinsundet is located on the east side of the island Leka in Lekafjorden. This is a deep fjord with depth of more than 200 m, and it is exposed to the open ocean towards the west. The domain is shown in blue in Figure 14, and the high-resolution mesh and topography is shown in Figure 18. The release point is located on the steep western slope of the fjord, over a depth of 120 m. The mesh for Jakobsteinsvika has high resolution in a larger area compared to the other fine scale meshes, because in Jakobsteinsvika we will also simulate release from a wellboat. The resolution at the release site and in the wellboat track is 20 m.

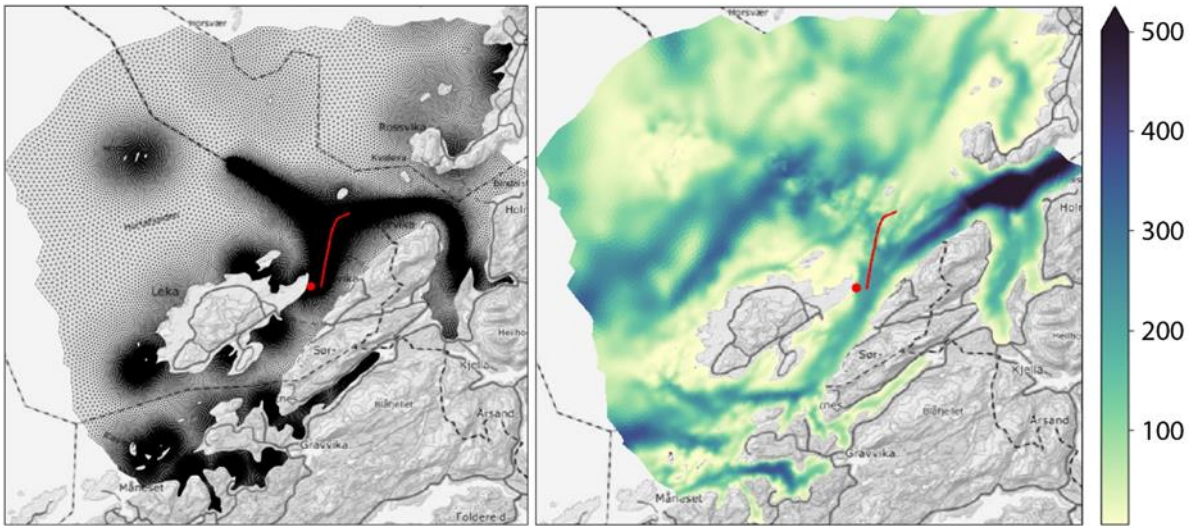

Figure 18. The mesh (left) and bottom topography (right) for Jakobsteinsvika. The red point shows the location of the release of H_2O_2 from fish cages, while the red line shows the locations of release from wellboat.

6.4 Circulation and stratification at the release sites

The circulation and stratification at the sites for release determines the spreading of H_2O_2. In Figure 19, the locations are shown together with the surface salinity field for August 19, 2014. It is clear that Kjelneset stands out as the most freshwater influenced location, as it is located in the outer parts of a fjord system that is heavily influenced by river runoff. Austvika is located inside a small fjord, while Indre Skjervøy and Jakobsteinsvika are located close to the open ocean.

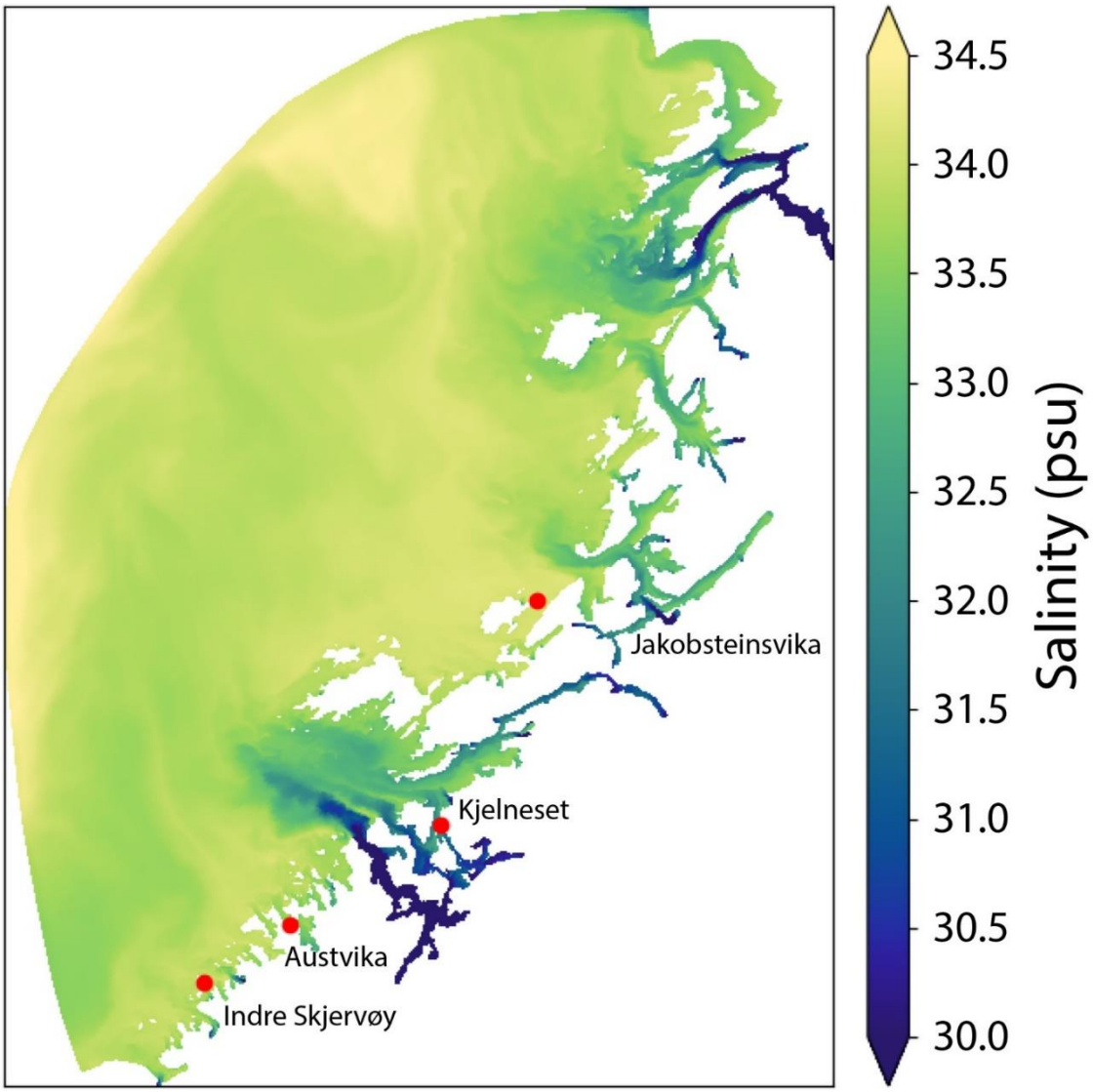

Figure 19. Modelled surface salinity at August 19, 2014. The sites for release of H_2O_2 is marked by red dots.

Figure 20-Figure 23 show the temperature, salinity and density at Indre Skjervøy, Austvika, Kjelneset and Jakobsteinsvika. The stratification varies strongly with season. The water masses are generally stratified in the summer months from May through September, while they are well mixed or have the weakest stratification in February and March. The strongest stratification is found at Kjelneset, which is stratified all year, and has the lowest salinities. This is due to its location near the mouth of the freshwater influences fjord system (Figure 19). The location with the weakest stratification is Jakobsteinsvika (Figure 23), which is near well mixed from October to May. The water columns at Indre Skjervøy and Austvika show a weak stratification from October to January/February, before it becomes well mixed in February and March.

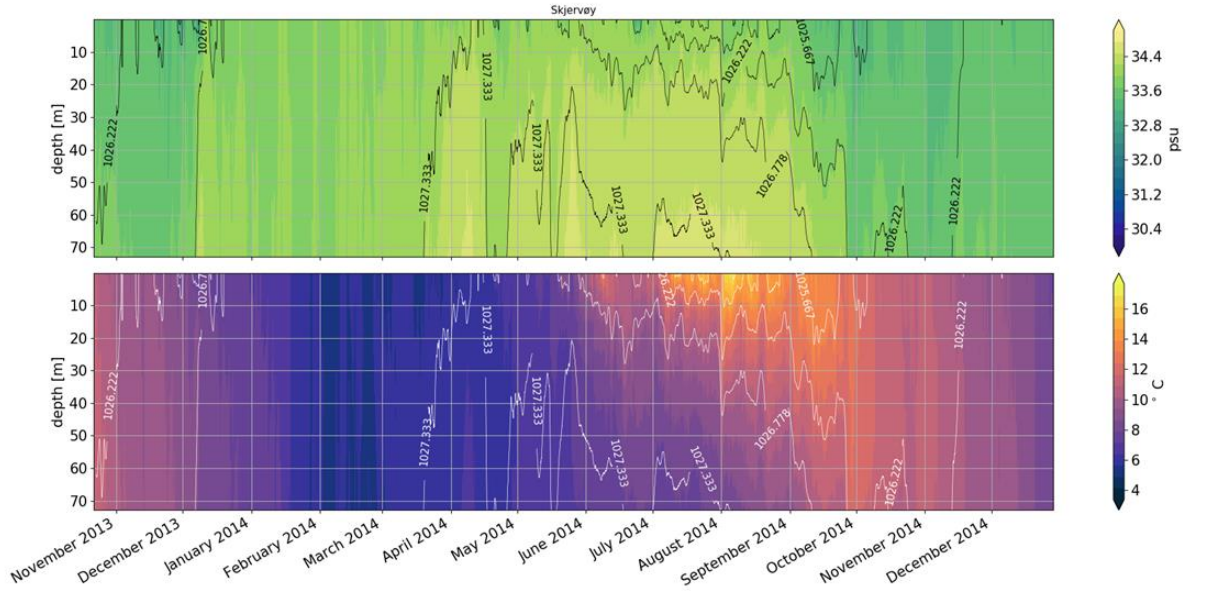

Figure 20. Salinity (upper), temperature (lower) and density (contours) at the release point at Indre Skjervøy. Stratification is strong were the density contours are close together.

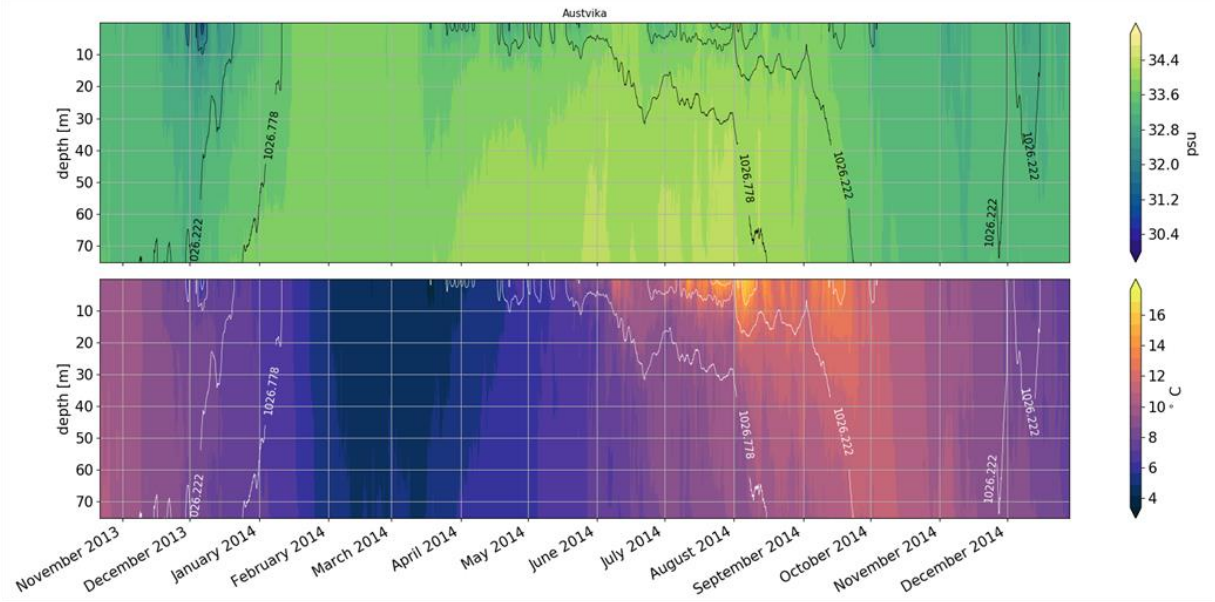

Figure 21. Salinity (upper), temperature (lower) and density (contours) at the release point at Austvika. Stratification is strong were the density contours are close together.

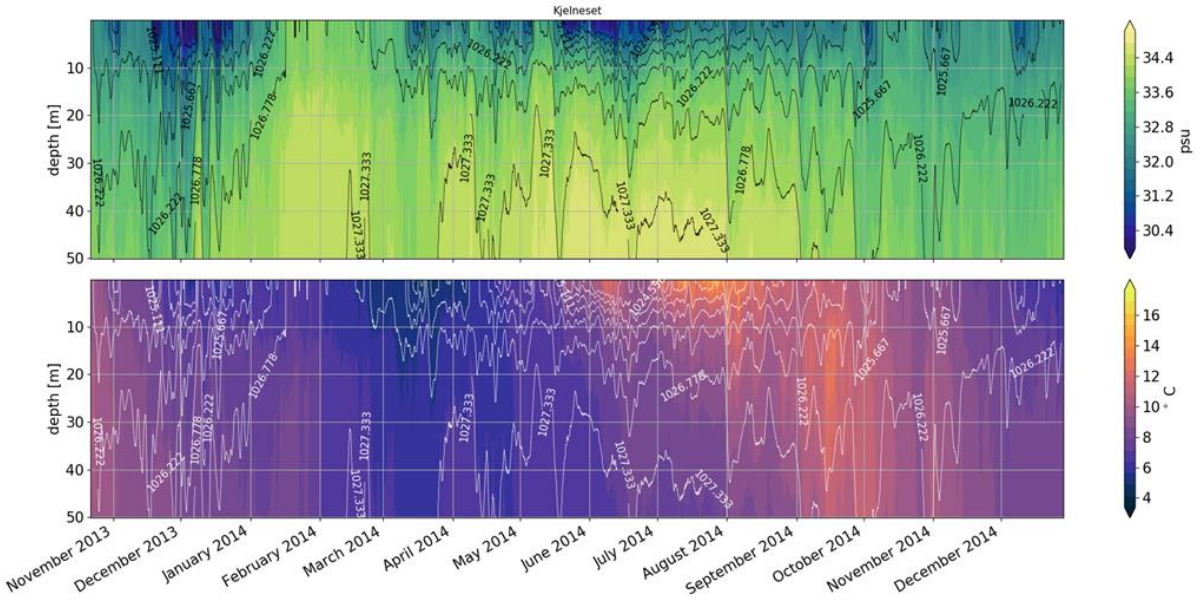

Figure 22. Salinity (upper), temperature (lower) and density (contours) at the release point at Kjelneset. Stratification is strong were the density contours are close together.

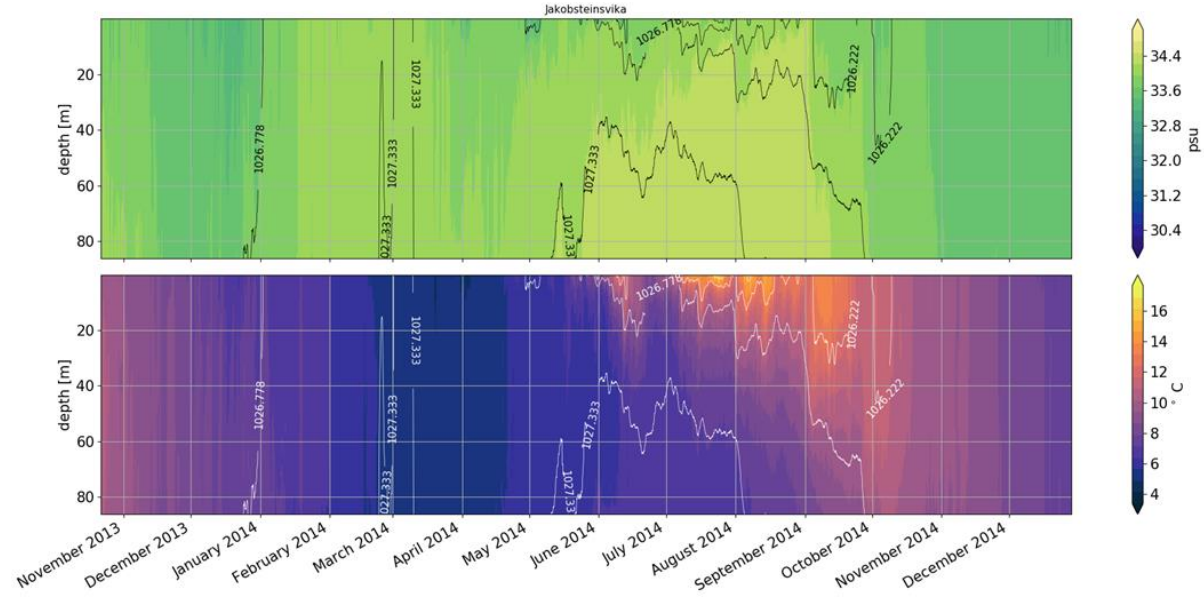

Figure 23. Salinity (upper), temperature (lower) and density (contours) at the release point at Jakobsteinsvika. Stratification is strong were the density contours are close together.

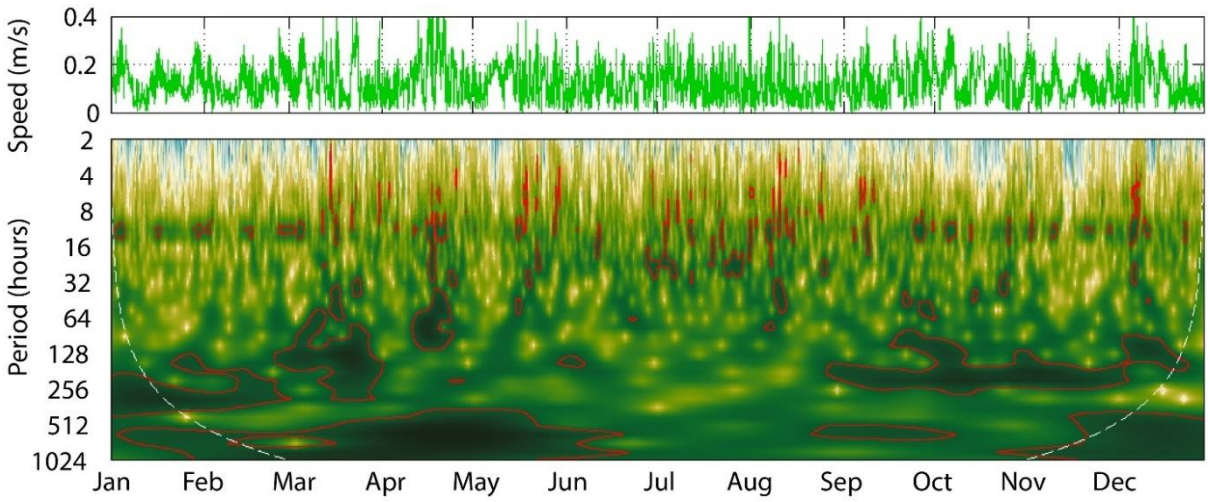

Figure 24. The surface layer current time series (top) and the wavelet power spectrum of the time series (below) for Indre Skjervøy.

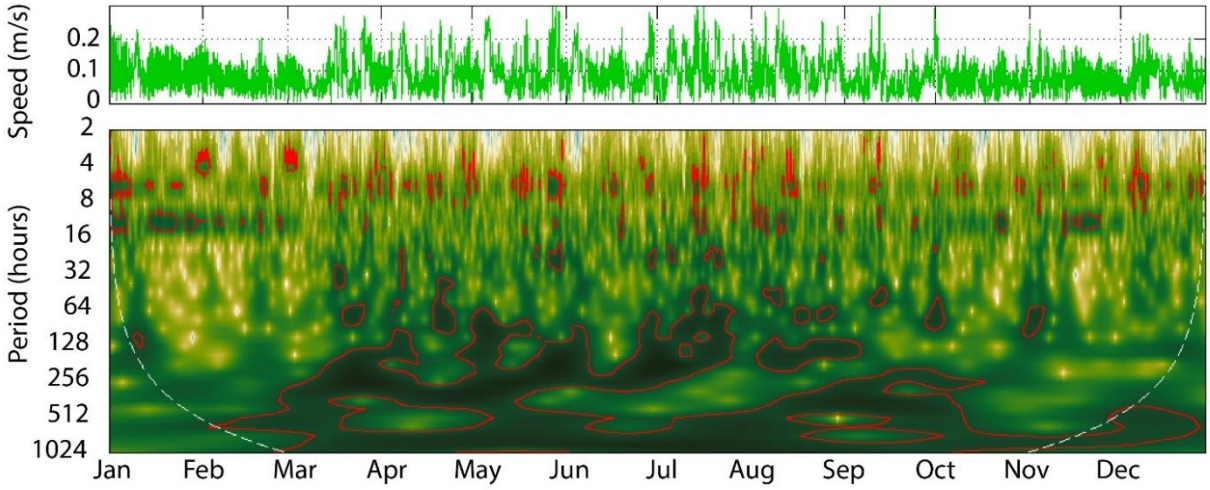

Figure 25. The surface layer current time series (top) and the wavelet power spectrum of the time series (below) for Austvika.

Figure 24- Figure 27 show the surface layer current and its wavelet power spectrum for Indre Skjervøy, Austvika, Kjelneset and Jakobsteinsvika. Kjelneset has the strongest currents, while Austvika has the weakest. Semi-diurnal tides are clearly visible in the power spectrum for all four locations. The most energetic currents vary on a period of semi-diurnal and shorter or two days and longer. Kjelneset stands out as it has less energy on the longer time-scales and more energy on the shorter time-scales than the other three locations. Harmful concentrations of H_2O_2 is only present for a few hours, and therefore high frequency is likely to be important in the spreading of H_2O_2. Since all locations have high energy on the shorter periods, we expect the initial direction of spreading from one location to have large variability.

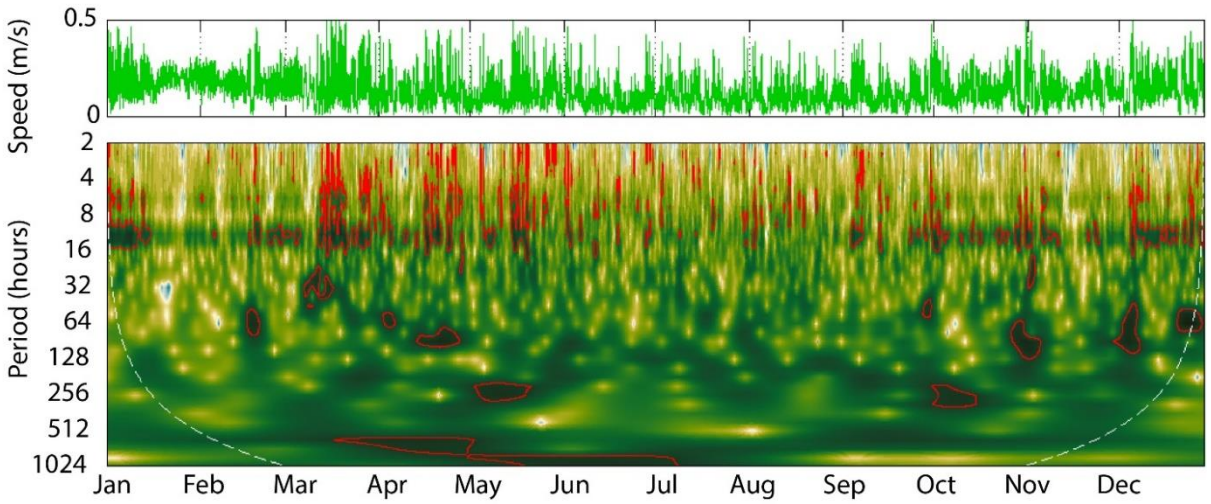

Figure 26. The surface layer current time series (top) and the wavelet power spectrum of the time series (below) for Kjelneset.

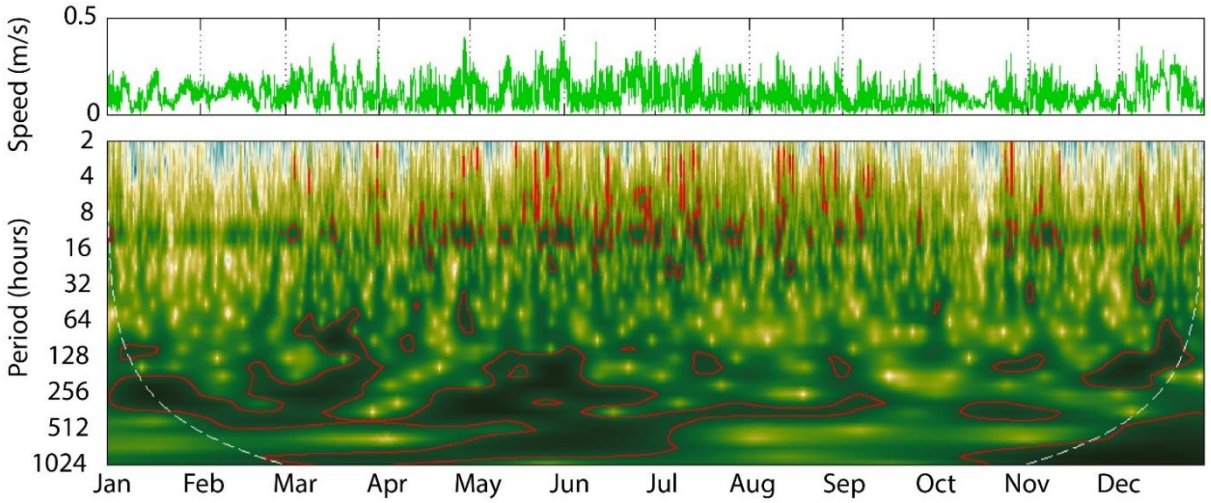

Figure 27. The surface layer current time series (top) and the wavelet power spectrum of the time series (below) for Jakobsteinsvika.

6.5 Modelling the spreading of H_2O_2 after delousing

During treatment in a fish cage, the fish net is usually raised to about 10 m depth, covered from beneath by a tarpaulin and H_2O_2 is mixed into the remaining seawater until the desired treatment dose is reached. After treatment, the tarpaulin is pulled away and the H_2O_2 is released into the ambient water. After treatment in a wellboat, the H_2O_2 is pumped into the sea. The volume contained in a wellboat varies. We have simulated a wellboat volume of 3 000 m^3 travelling at a speed of 7 knots. After delousing, this volume of the seawater and H_2O_2 mixture is typically pumped into the sea during 45 minutes.

For delousing treatment the dose of H_2O_2 is dependent on temperature, and in the modelling, we follow the industry standard in Norway (https://www.felleskatalogen.no). For temperatures below 4 °C, the treatment concentration is 2.1 g/l, for the temperature range of 4-8 °C the

treatment concentration is 1.8 g/l, and for temperatures exceeding 8 °C, a treatment concentration of 1.5 g/l is used.

During delousing in fish cages, the volume enclosed by the tarpaulin is measured, and we use these volume data to initialize the model experiments.

6.5.1 Experiments

Modelling were done for fish cages with 120 m and 160 m circumference. Normally, a fish farm has several fish cages located next to each other, and therefore a delousing might include several fish cages. The release from a single fish cage and from fish farms of four cages were modelled. Whether the release is from fish cages or wellboats, releases throughout the whole year were simulated.

6.5.1.1 Single releases

Single releases from 120 m and 160 m fish cages were modelled. This was done by simulating 48 releases evenly spread throughout the year. This was done for all four model sub-regions. In these simulations the maximum volume observed during delousing (14 900 m^3 for 120 m and 21 000 m^3 for 160 m) was used.

6.5.1.2 Multi-cage releases

Delousing of a fish farm often includes delousing of several fish cages. The release from four fish cages during two days was modelled. Thus, one treatment consists of four single releases in a two-day period. In these scenarios, releases from 120 m cages were simulated. This is done one time per month throughout one year. Maximum observed volume (14 900 m^3) is used for all locations, but Jakobsteinsvika was run an extra time with the average observed volume for 120 m cages (7 700 m^3). The reason for the extra run at Jakobsteinsvika is that at this location the results were compared with the releases from wellboat.

6.5.1.3 Release from wellboat

The release from wellboat was setup to resemble the delousing of four fish cages. This requires eight releases from the wellboat. As in the multi-cage releases, this was done one time per month. The volume of the wellboat that was simulated was 3 000 m^3.

6.5.2 Initialization and setup of model experiments

Simulating the release of H_2O_2 from a fish cage is in principle very simple. H_2O_2 can be represented by a tracer and the tracer concentration within the fish cage used as an initial condition. The release from a wellboat is a little more complicated as the wellboat is moving while pumping the H_2O_2 into the sea. In this case, a flux of H_2O_2 from a moving source was simply specified. However, H_2O_2 is denser than seawater, and using the simple approach requires using a model that can realistically represents the sinking from the initial release locations.

The density of pure H_2O_2 is 1 450 kg/m^3. It was assumed that the density of the mixture of H_2O_2 and seawater is given by a linear mixing of the two densities. Using 2 g/L of H_2O_2 in seawater, which is a normal concentration for sea lice treatment, gives an increase in density of about 0.6 per mill. If the mixture would simply accelerate downward with an acceleration equal to the reduced gravity given by this density increase, it will reach 100 m depth in three minutes.

Modelling the sinking of H_2O_2 requires non-hydrostatic dynamics. FVCOM does include a non-hydrostatic module (Lai 2010), and this has been used to investigate the sinking of the H_2O_2 from fish cages. This shows that H_2O_2 released from a fish cage sinks and reaches 100 m in 7.5 minutes in homogeneous water. In stratified water, the sinking is equally fast, but stops when the H_2O_2 reaches ambient water of similar density. This supports the conclusion that the H_2O_2 will sink until it reaches its density level and that the sinking will occur only a few minutes after release.

The non-hydrostatic module is computationally very demanding, and it is therefore not practical for us to use this in all the model experiments run in this work. Forty-eight single releases from 120 m and 160 m fish cages at four locations sums up to 384 simulations. The multi-cage releases consist of 4 single releases and simulating 12 multi-cage releases per year in four locations sums up to 192 simulations. Running the non-hydrostatic model for all these simulations is simply too much. However, the non-hydrostatic dynamics is only needed when the sinking occurs, which is in the first few minutes after release. Using this fact, we have constructed a simplified model for the sinking of H_2O_2. This model gives the depth and concentration after sinking, which is used to initialize the hydrostatic version of FVCOM, in order to simulate further horizontal spreading.

6.5.2.1 A parameterization of the sinking of H_2O_2 from fish cages

Our aim is to investigate the spreading of H_2O_2 over timescales of hours and days. Since the sinking is happening during the first few minutes, this is treated in the initialization of the model. Based on the stratification at the time of release, the sinking depth and the mixing that occurs during sinking was calculated. The result of this is used to initialize the model.

The calculations of sinking and mixing during the initial sinking state, is based on chapter 10 in Cushman-Roisin (2018). It was assumed that the H_2O_2 mixture released from a fish cage will behave like a thermal described by Cushman-Roisin (2018). The model of sinking of H_2O_2 is based on three conservation equations: Conservation of volume, momentum and density deficiency. It models the evolution of the average properties of the released mixture of H_2O_2 and seawater.

A closer description of this model is presented in Appendix 1 (chapter 11). As the mixture sinks, it entrains ambient water decreasing the concentration. The model calculated the depth to which the mixture sinks and its volume and H_2O_2 concentration at this depth. The calculated values are used to initialize the H_2O_2 concentrations in the hydrostatic model. Results showing the seasonal variation of the initial sinking of H_2O_2 are presented in Figure 31-Figure 34**.**

6.5.2.2 Release of H_2O_2 from wellboats

The delousing of a fish farm consisting of four fish cages was modelled using a wellboat performing two treatments per fish cage with a release H_2O_2 eight times per delousing, which was simulated one time per month. The release from the wellboat is not suited to be modelled using the type of simplified dynamics described above since the boat is in movement and since the release is continuous. For this reason, the non-hydrostatic FVCOM was chosen to model the initial sinking after release from a wellboat.

6.5.2.2.1 Modelling sinking of H_2O_2 released from a wellboat

To model the initial sinking, it was decided to set up an idealized domain to do simulations using the non-hydrostatic FVCOM. The idealized domain is rectangular, 4000x2000 m, and 100 m meter deep. Horizontal resolution is 2 m, and vertical resolution is 1m. One time per

month was chosen, representing the monthly delousing, and transfer the stratification from a point representing the wellboat track in the Jakobsteinsvik model, to the idealized model, one for each time. The idealized model is horizontally homogeneous. The setup is used to do one simulation per month to simulate initial sinking after release from a wellboat. The sinking of the H_2O_2 is the only forcing driving circulation in the idealized model.

The tracer concentration in the wellboat diminish with time during release, since ambient water is pumped back into the well at the same rate as water containing H_2O_2 is pumped out. Let C be the H_2O_2 concentration at any given time and C_0 be the initial concentration. Let V_f be the volume flux out/in of the boat and V be the volume of the tank the tracer is contained in minus the volume occupied by the fish. The H_2O_2 concentration in the ship can then be expressed as $C(t) = C_0 e^{-\frac{V_f}{V}t}$, where $V_f = 2.23\,{}^{m^3}/_{s}$ and $V = 960m^3$.

The wellboat track is represented by a list of nodes during release. H_2O_2 is released into the idealized model by representing a wellboat moving along the track for 15 minutes. The model was ran until the water had settled at its equilibrium depth. Figure 28 shows the maximum concentration at any depth in the idealized model, after equilibrium is reached. In the wellboat, there is three tanks of 960 m^3 each, and during one release, the contents of all three tanks are released into the sea, one at a time.

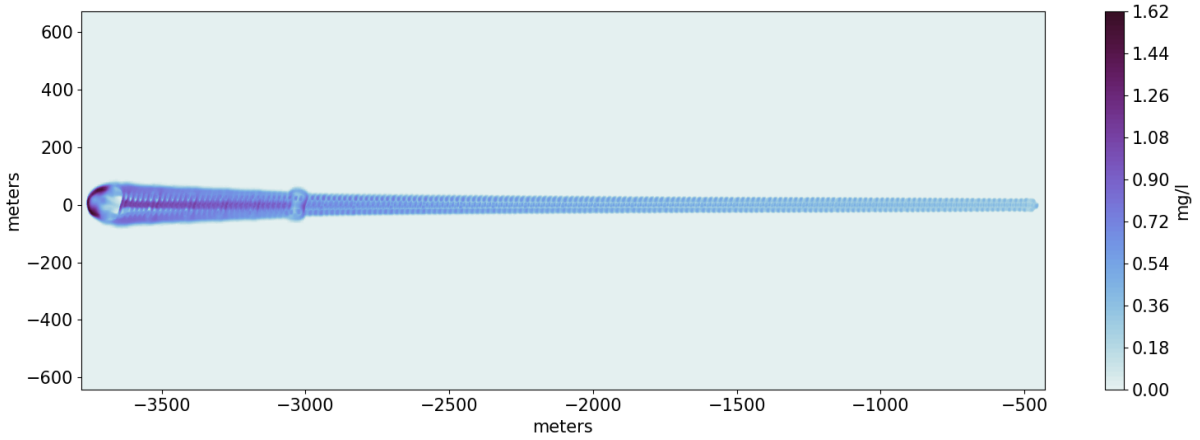

Figure 28. The maximum concentration at each grid point after release and sinking to the equilibrium depth in the idealized model.

The grid used for non-hydrostatic modelling is idealized and bears no resemblance to the one used by the hydrostatic model to model horizontal spreading since the horizontal resolution is much coarser ($\sim 2m\ vs\ \sim 20m$) and the depth varies with bathymetry. The ship runs the same distance, and based on the distance from the initial location of the ship, we identified the nodes in the hydrostatic model which corresponded to the ones in the non-hydrostatic model, found the depths that corresponded, and used these to assign a concentration to all nodes in- and around the ship track. Figure 29 shows the initial field in the hydrostatic model, created by transferring fields from the idealized non-hydrostatic model.

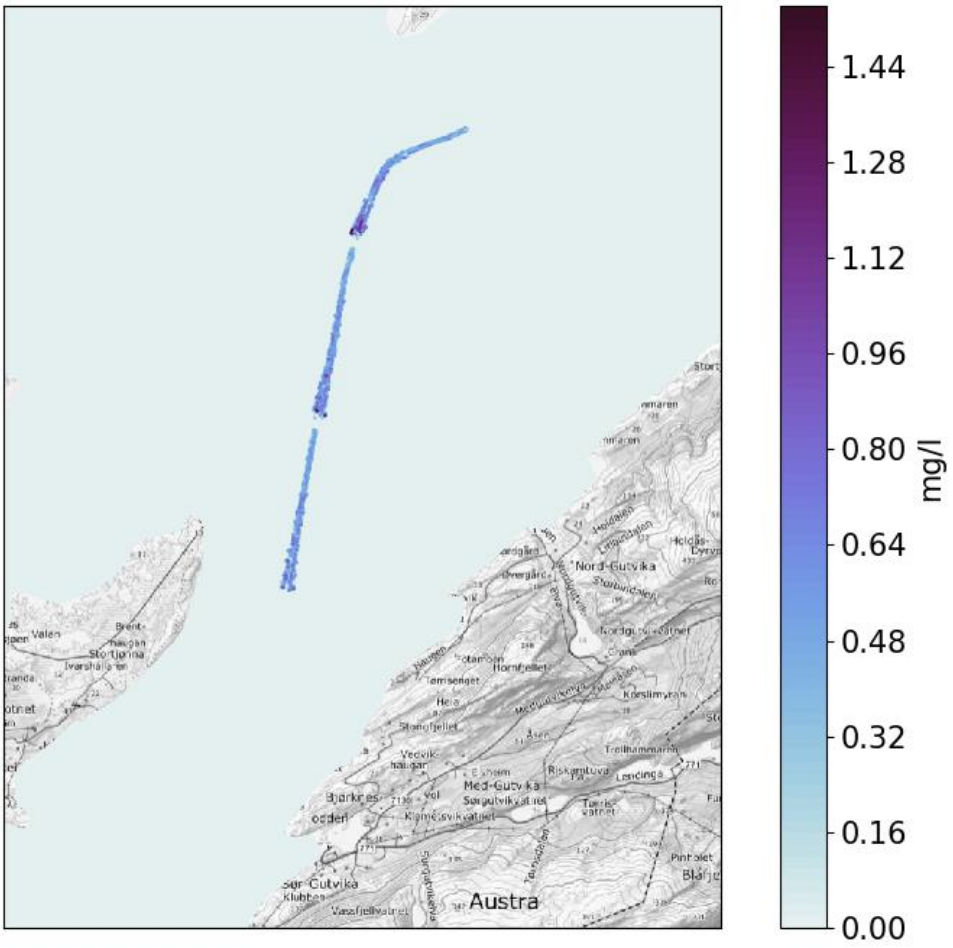

Figure 29. The initial vertical maximum concentration in the hydrostatic model.

Each cell in the hydrostatic model grid covers a bigger volume than its non-hydrostatic counterpart due to its greater grid size. We therefore multiply the H_2O_2 concentration in the hydrostatic model with a weight *"w"* to ensure conservation of mass.

Figure 30 shows the vertical distribution of the tracer concentration along the ship track in the non-hydrostatic model on the top-panel and in the hydrostatic model in the bottom-panel after data transfer for the modelled stratification in May 2014. Notice that the concentration in the hydrostatic model is lower, to conserve mass.

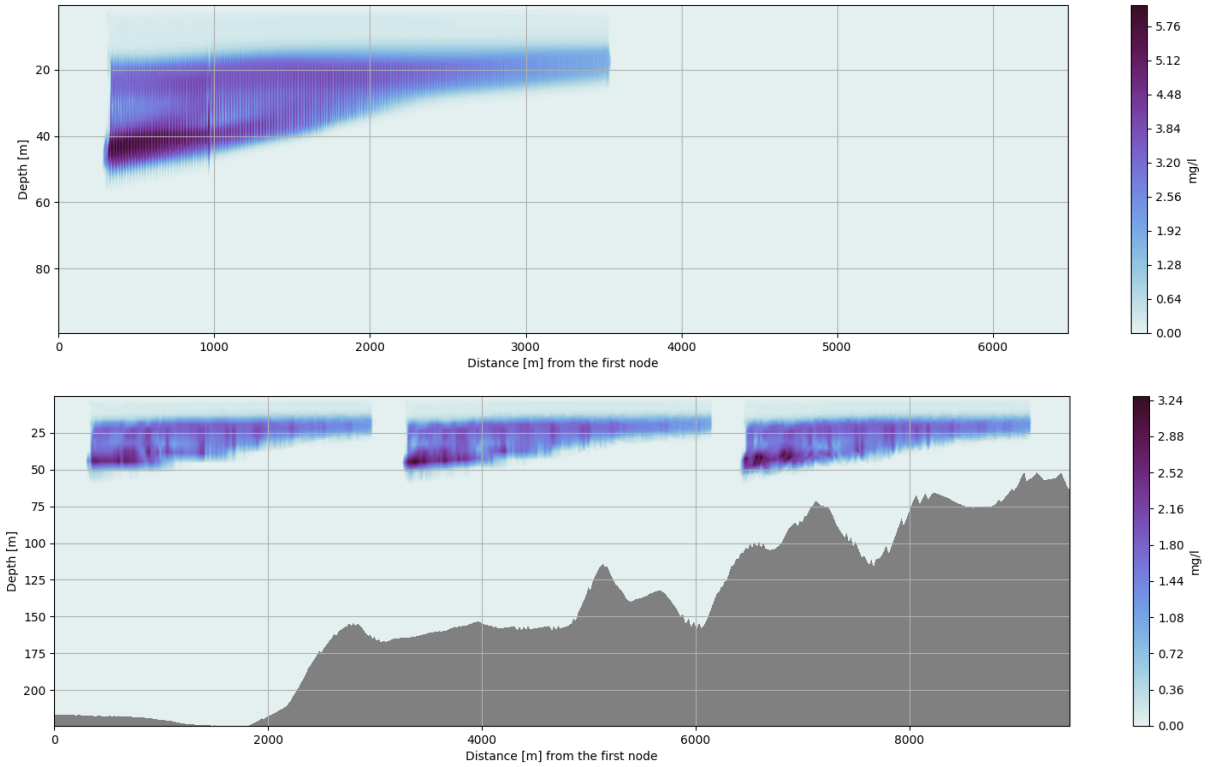

Figure 30. H_2O_2 concentrations along the ship track in May. Idealized model (upper) and hydrostatic model (lower).

6.6 Results

6.6.1 Initial sinking of H_2O_2

6.6.1.1 Release from fish cages

The initial sinking of H_2O_2 released from fish cages, is calculated by the parameterization presented on page 52. Here, we present results from running the parameterization on seasonal values of the stratification. Figure 31 to Figure 34 show the sinking depth and concentrations after sinking for a 120 m fish cage, and the differences between a 120 m and a 160 m fish cage for all four locations. At the locations Indre Skjervøy and Jakobsteinsvika, the H_2O_2 from both type of cages sinks to the bottom in autumn, winter and spring months. At Austvika H_2O_2 sinks to the bottom in February and March, while at Kjelneset the stratification prevents sinking to the bottom also in the winter months. Where and when the H_2O_2 sinks to the bottom, the concentration after sinking depends on the depth, but in our cases it is between 200 and 300 mg/l for the 120 m cage. At the same time, there is no difference in sinking depth between the 120 m and the 160 m fish cages, and the difference in H_2O_2 concentration between the two is constant with values around 60 mg/l.

The difference in sinking depth between the release from 120 m and 160 m fish cages is also small in summer when the stratification is strong. Between June and September this difference is below 2 m for all four locations. The concentration is high when the sinking depth is small, and in June the concentration after sinking is around 1500 mg/l. The concentration difference between release from 120 m and 160 m fish cages varies, but it is mostly between 20 and 60 mg/l.

The difference in sinking depth between release from a 120 m and a 160 m fish cage is largest in the periods of transition between well mixed winter conditions and strongly stratified summer conditions. For Indre Skjervøy, Austvika and Jakobsteinsvika, this occurs in April-May, and in December. For Kjelneset, which is never well mixed, the largest depth differences is found in February, when the stratification is at its weakest. When the difference in sinking depth is large, the difference in concentration is at its smallest.

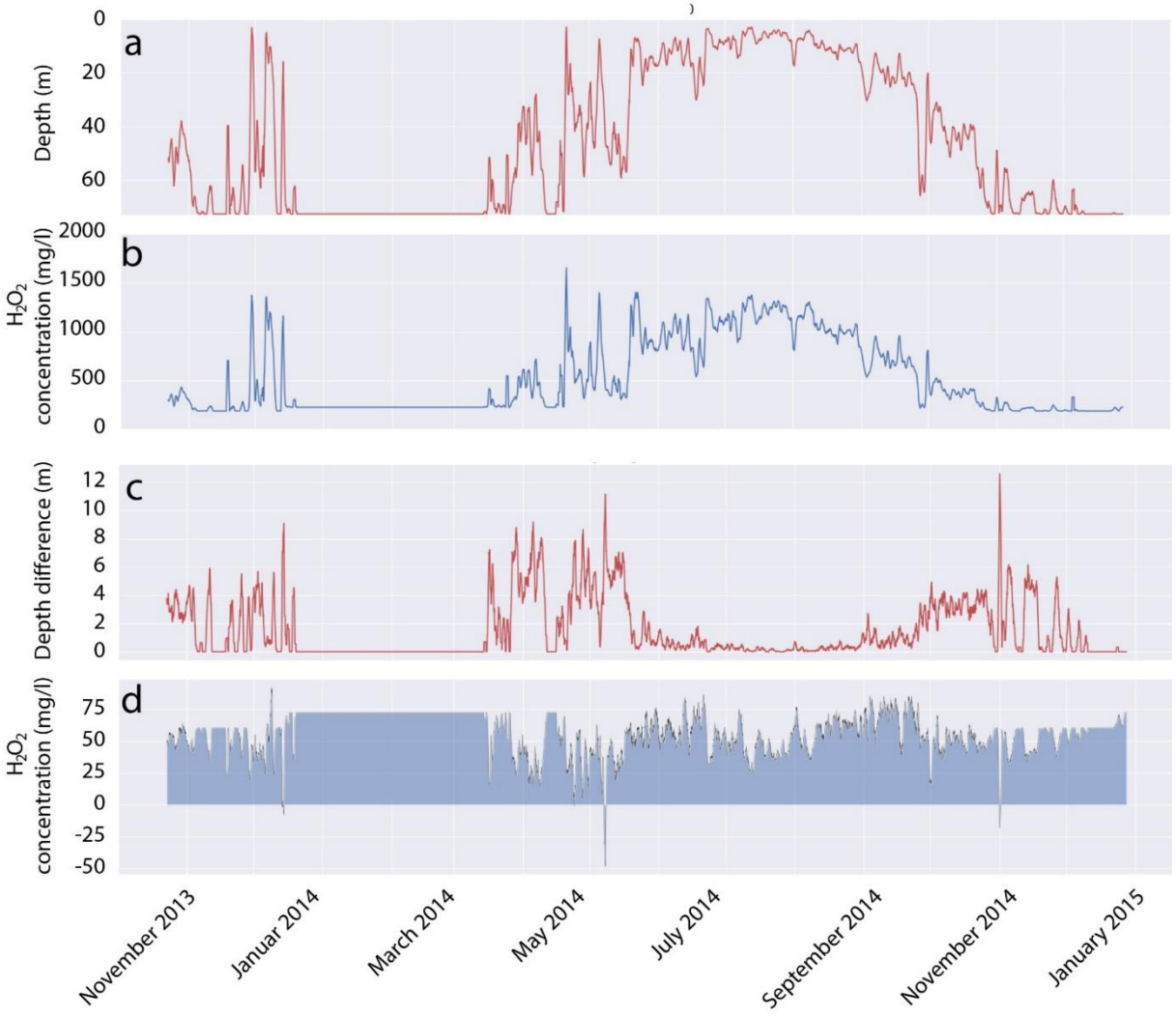

Figure 31. Indre Skjervøy: a) Sinking depth of H_2O_2 after delousing in a 120 m fish cage, b) the H_2O_2 concentrations after sinking, c) difference in sinking depth and d) difference in concentration between release from a 160 m and a 120 m fish cage (160 – 120).

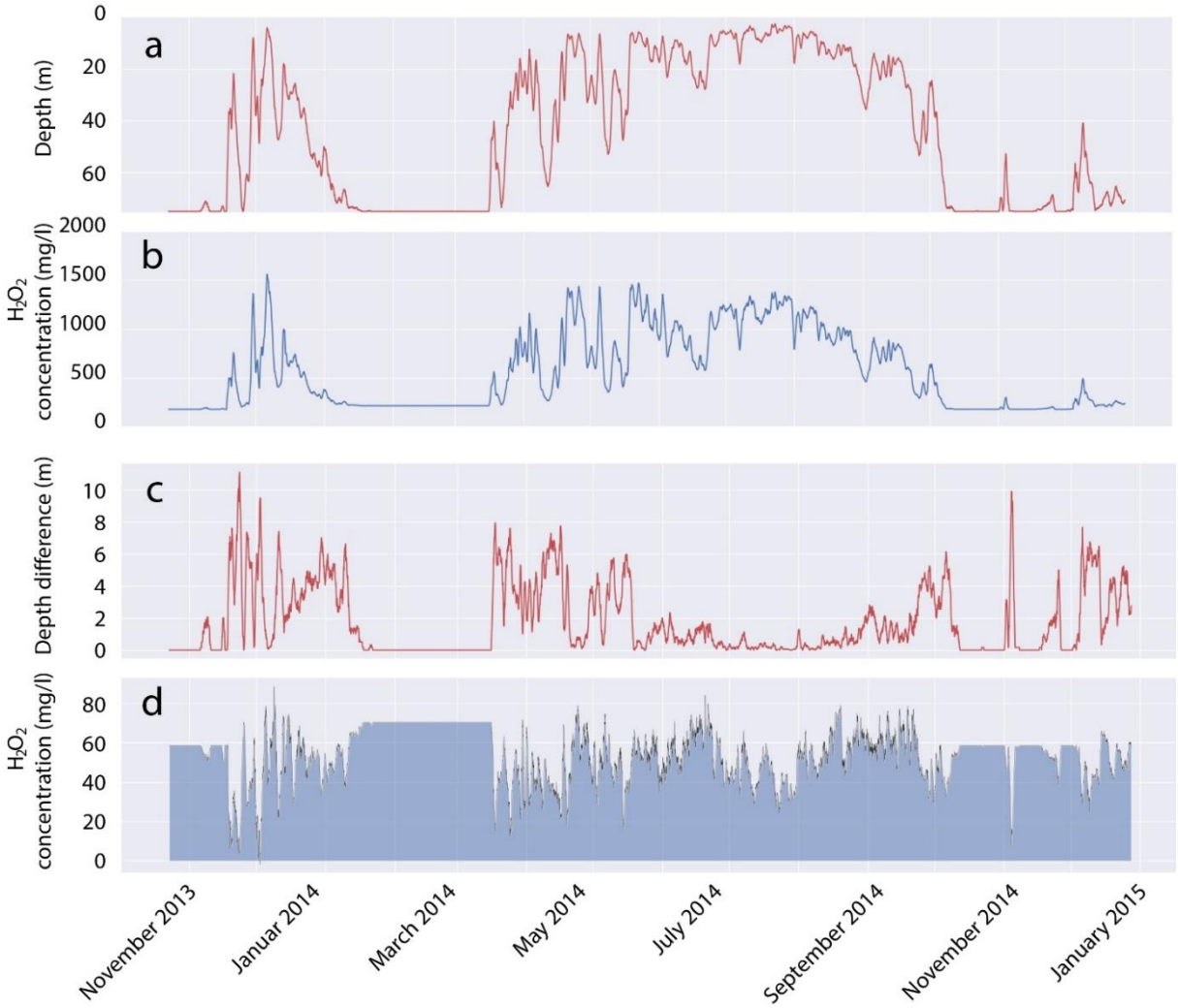

Figure 32. Austvika: a) Sinking depth of H_2O_2 after delousing in a 120 m fish cage, b) the H_2O_2 concentrations after sinking, c) difference in sinking depth and d) difference in concentration between release from a 160 m and a 120 m fish cage (160 – 120).

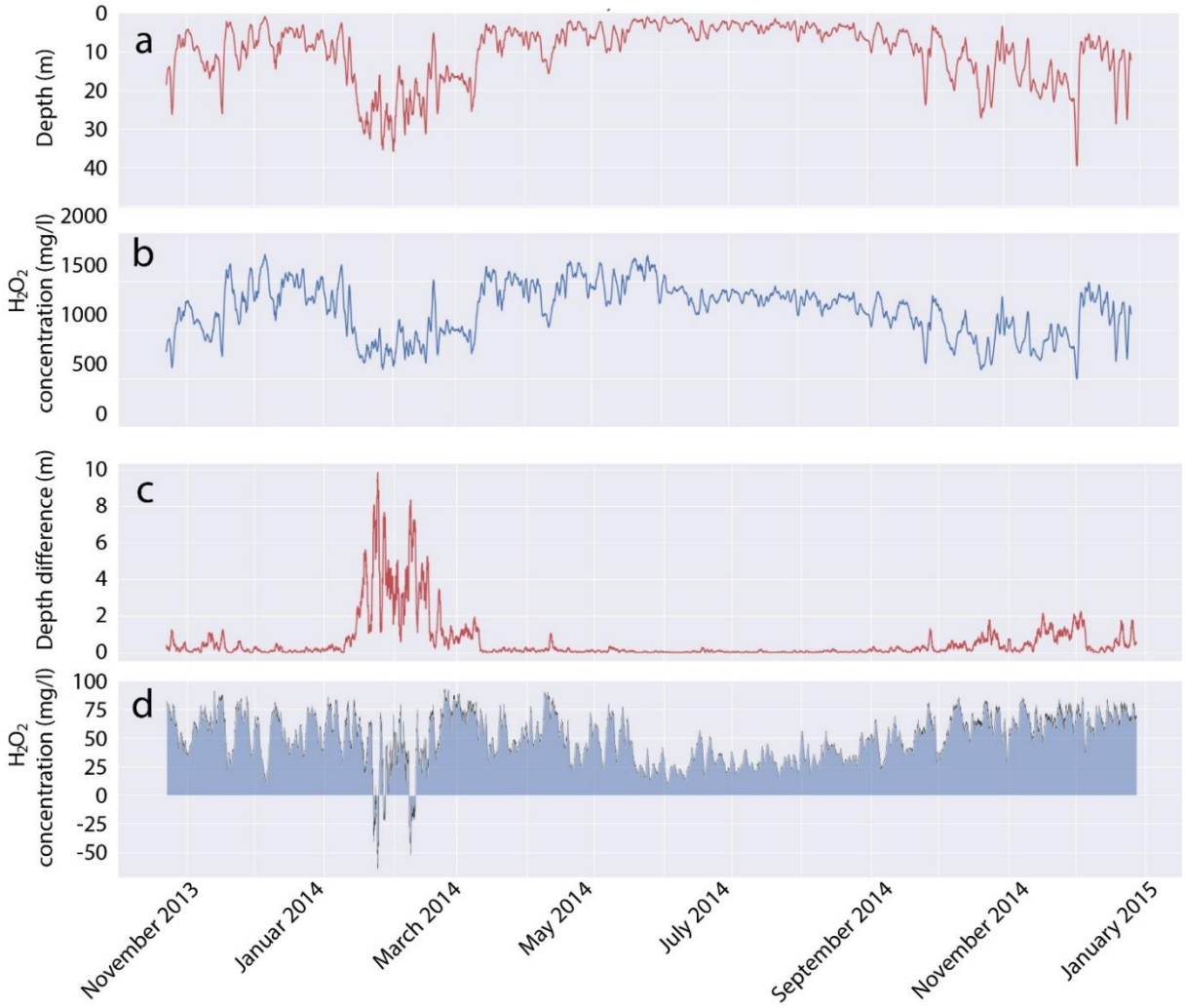

Figure 33. Kjelneset: a) Sinking depth of H_2O_2 after delousing in a 120 m fish cage, b) the H_2O_2 concentrations after sinking, c) difference in sinking depth and d) difference in concentration between release from a 160 m and a 120 m fish cage (160 – 120).

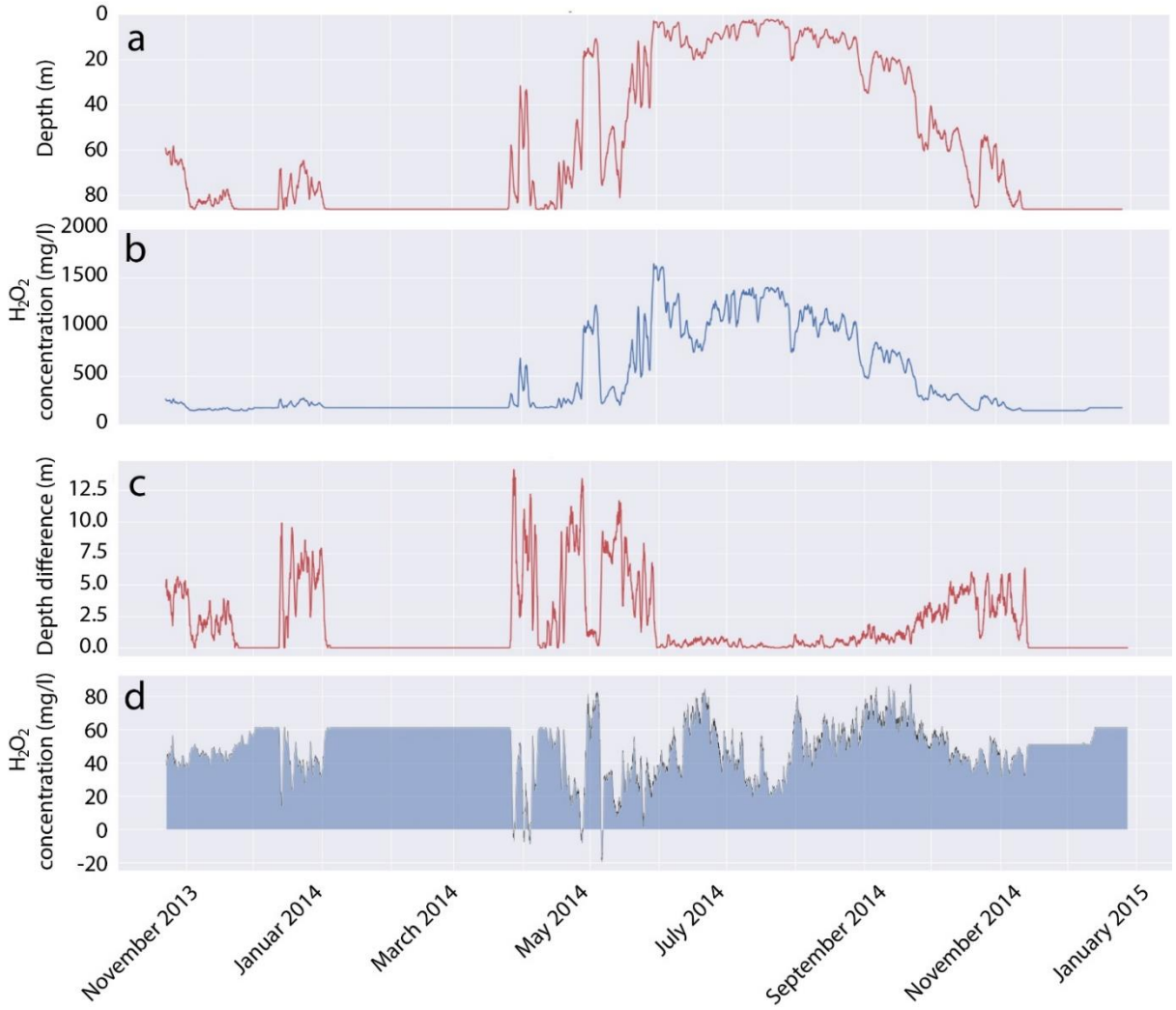

Figure 34. Jakobsteinsvika: a) Sinking depth of H_2O_2 after delousing in a 120 m fish cage, b) the H_2O_2 concentrations after sinking, c) difference in sinking depth and d) difference in concentration between release from a 160 m and a 120 m fish cage (160 – 120).

6.6.1.2 Release from wellboats

The sinking of H_2O_2 from a wellboat is simulated using the non-hydrostatic FVCOM, as described on page 52. The results are presented in Table 10. The sinking depths are comparable to the sinking depth from fish cage at Jakobsteinsvika (Figure 34). The concentration after sinking when released from wellboat, is in the order of 100 times lower than the concentration after sinking when released from a fish cage (comparing Table 10 with Figure 34 b).

Table 10. The depth to which the release of H_2O_2 from the wellboat reached [m], and the concentration [mg/l]. The depth is measured by visual inspection, concentration based on NH-model results.

Month	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Des**
Depth	30	75	100	30	50	30	20	15	15	15	40	100
Concentration	9.5	2	1.5	9	5.5	7	18	18.5	16	16	7	1

6.6.2 Single releases from fish cages

To investigate the differences in using 120 m or 160 m fish cages, we again compare the H_2O_2 concentrations resulting from delousing in the two types of cages. The maximum concentrations over all depths at each horizontal point experienced in all the 48 runs are shown in Figure 35-Figure 38, where the 160 m cage releases are shown in the left panels (A) and the difference between 160 m and 120 m releases are shown in the right panels (B). A general observation from all four locations is that concentrations up to about 300 mg/l can occur up to about 1 km from the release site, while 10 mg/l can occur ~5km from the release. Release from a 160 m fish cage will give higher concentrations than release from a 120 m cage. A difference between 1 mg/L and 10 mg/L can occur several km's away from release point. A difference between 10 mg/L and 50 mg/L typically occurs from one to four km's from the release site, while differences of a few hundred mg/L occurs within one km from the release. Differences above 500 mg/L typically occurs right at the release point.

Comparing the locations in Figure 35-Figure 38, it can be seen that Kjelneset to some degree stands out, with a more evenly distributed directional spread of the maximum concentrations. This difference is not surprising since the sinking depth is shallow compared to the other locations (Figure 33). The shallower sinking leads to less dilution before the H_2O_2 is spread horizontally by the current. Furthermore, the spreading in the shallower layers is more affected by the wind-driven component of the current, which typically has more directional spread than the tidal component.

For organisms, it is not only the concentration of H_2O_2, but also the exposure time that determines how harmful a plume is. Illustrations of the average number of hours the single releases result in concentrations above 1 mg/L and 10 mg/L are shown in Figure 39-Figure 42 and Figure 43-Figure 46, respectively. Also, in these figures, the 120m releases and the 160m releases are compared (B). For both the 1 mg/L and 10 mg/L concentrations there is a difference in exposure times between the locations. Austvika is the location with the longest exposure times with an average of 6.4 hours above 1 mg/L and 1.75 hours above 10 mg/L right next to the release. This is followed by Kjelneset with corresponding values of 4.8 and 1.6 hours, while the more exposed locations further out have shorter exposure times (4.0 and 1.4 hours for Indre Skjervøy; 3.2 and 1.4 for Jakobsteinsvika).

To illustrate how the exposure times vary in the vertical, the average number of hours is plotted as a function of distance from the release points in Figure 47-Figure 50 (1 mg/L) and Figure 51-Figure 54 (10 mg/L). Since there is little sinking at Kjelneset (see Figure 33), this location also stands out with higher exposure times near the surface. For the other locations, the highest exposure times are typically found below the surface at depths between 25 – 100 m. The more sheltered location, Austvika and Kjelneset, on average have higher exposure times of concentrations above 1 mg/L. However, for concentrations above 10 mg/L the difference is small.

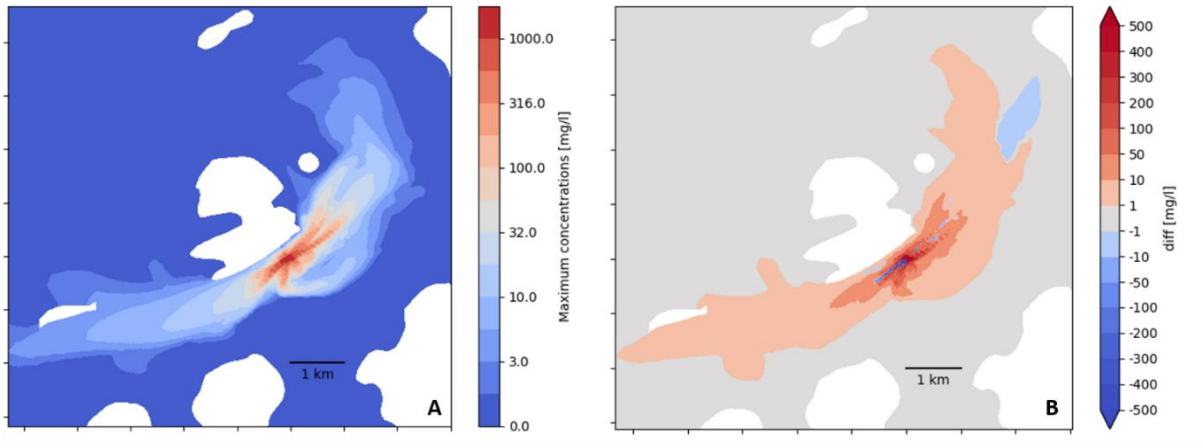

Figure 35. Maximum concentration from all simulated single releases at Indre Skjervøy. Panel A shows releases assuming 160 m circumference cages (initial volume of 21 000 m^3). Panel B shows the difference between releases assuming 160 m- and 120 m circumference cage (initial volume of 14 900 m^3).

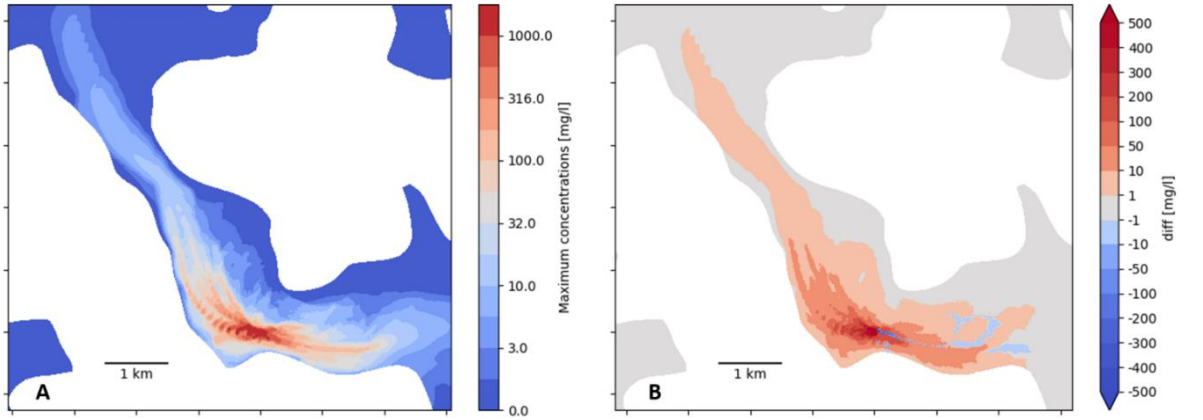

Figure 36. Maximum concentration from all simulated single releases at Austvika. Panel A shows releases assuming 160 m circumference cages (initial volume of 21 000m^3). Panel B shows the difference between releases assuming 160 m- and 120m circumference cage (initial volume of 14 900 m^3).

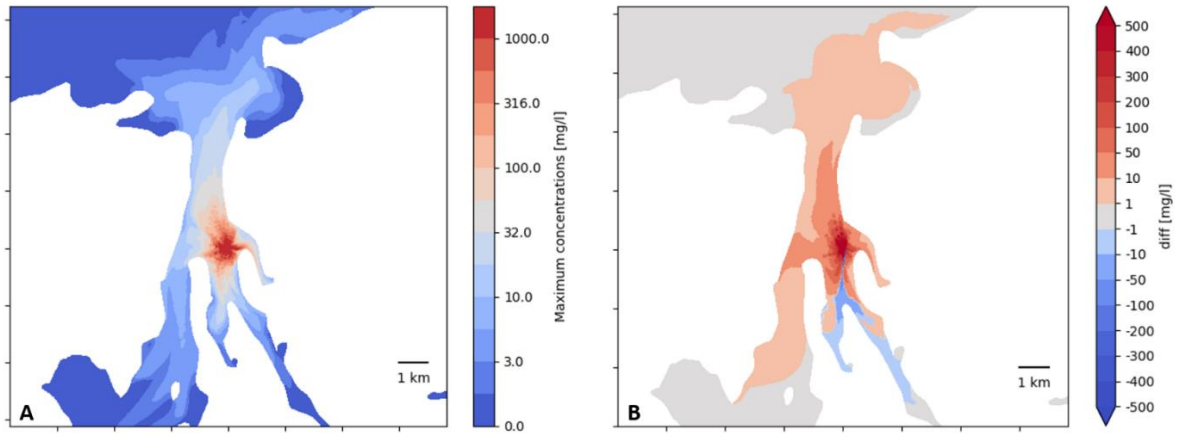

Figure 37. Maximum concentration from all simulated single releases at Kjelsneset. Panel A shows releases assuming 160 m circumference cages (initial volume of 21 000 m^3). Panel B shows the difference between releases assuming 160 m- and 120 m circumference cage (initial volume of 14 900 m^3).

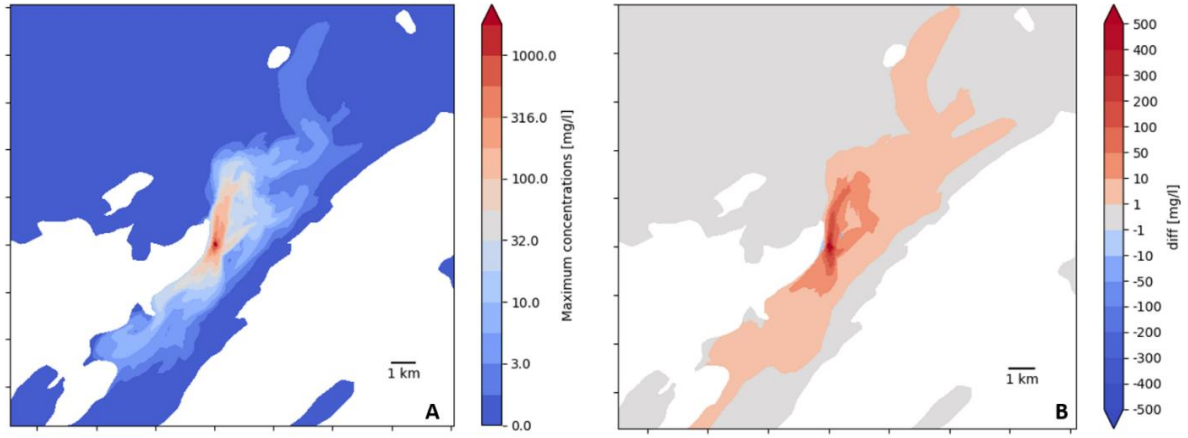

Figure 38. Maximum concentration from all simulated single releases at Jakobsteinsvika. Panel A shows releases assuming 160 m circumference cages (initial volume of 21 000 m^3). Panel B shows the difference between releases assuming 160 m- and 120 m circumference cage (initial volume of 14 900 m^3).

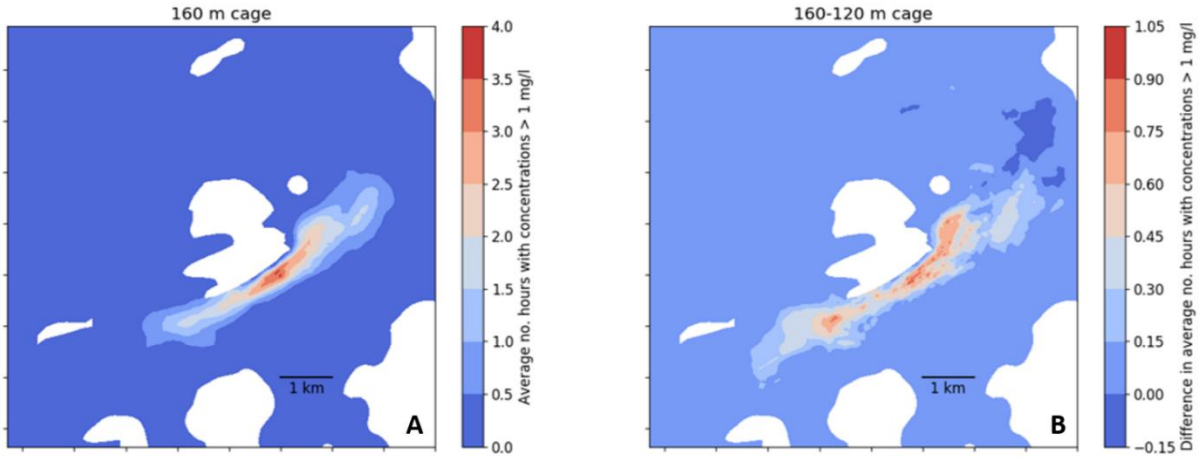

Figure 39. Average number of hours that a release at Indre Skjervøy results in concentrations above 1 mg/L for a release from a 160m cage (A), and the difference between a 160- and a 120 m cage.

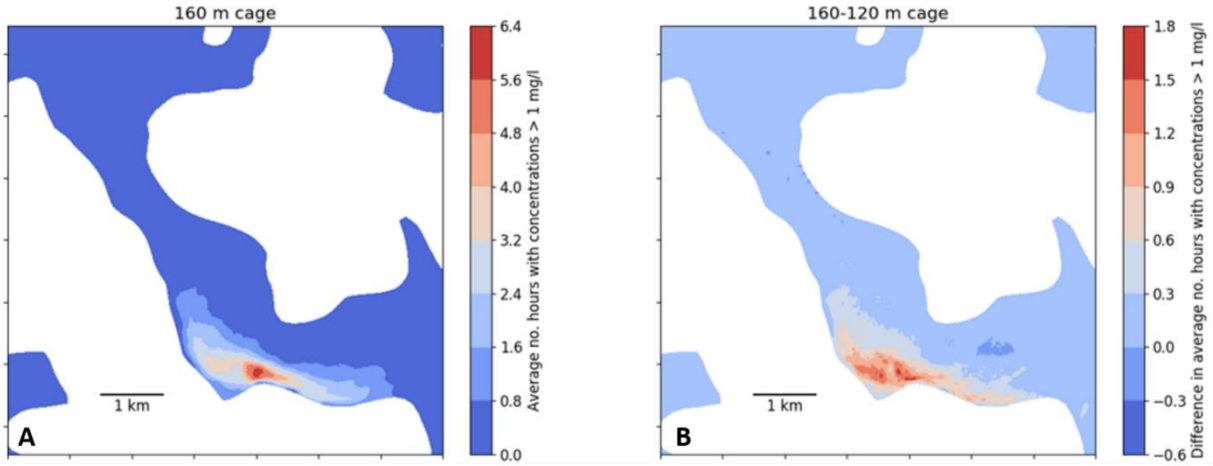

Figure 40. Same as Figure 39 for Austvika.

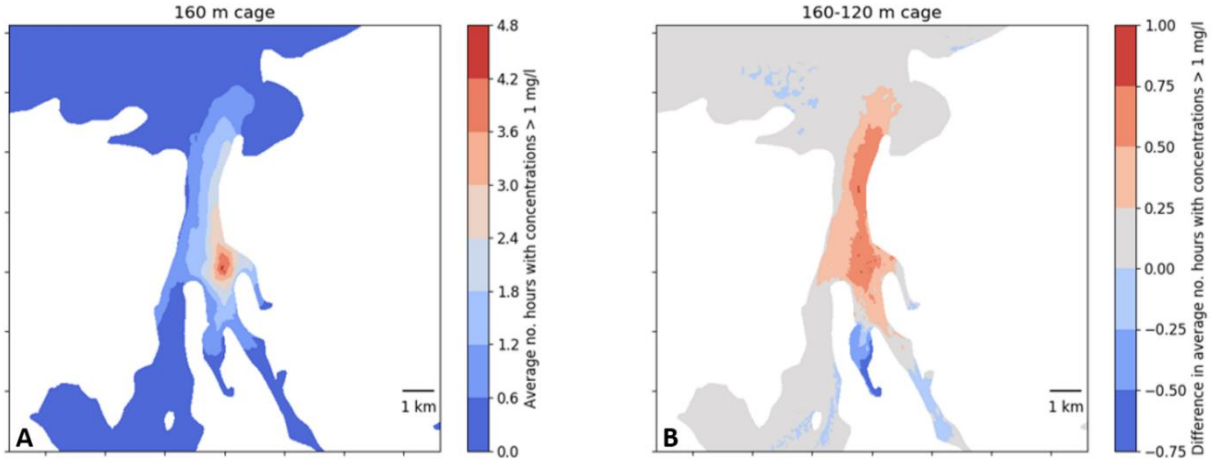

Figure 41. Same as Figure 39 for Kjelneset.

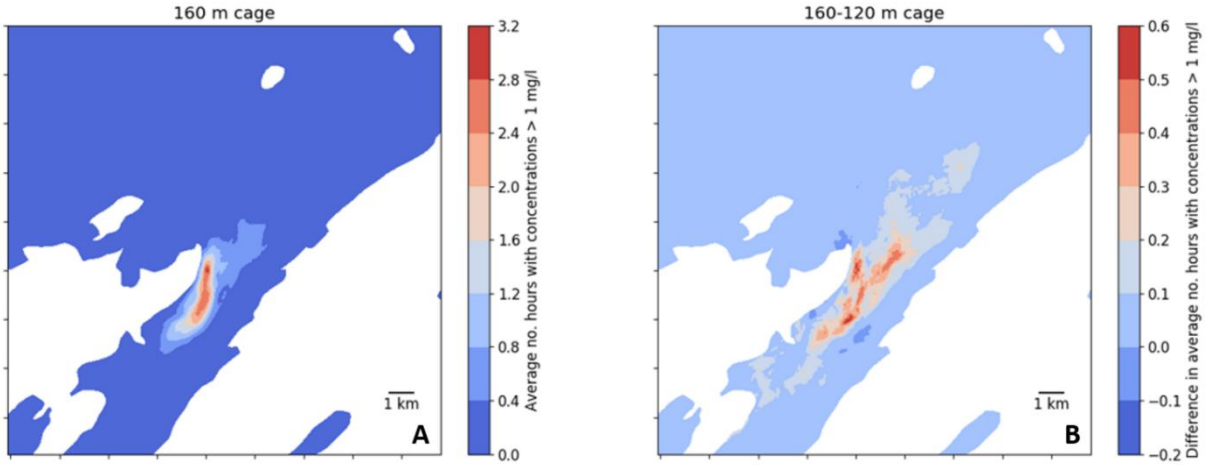

Figure 42. Same as Figure 39 for Jakobsteinsvika.

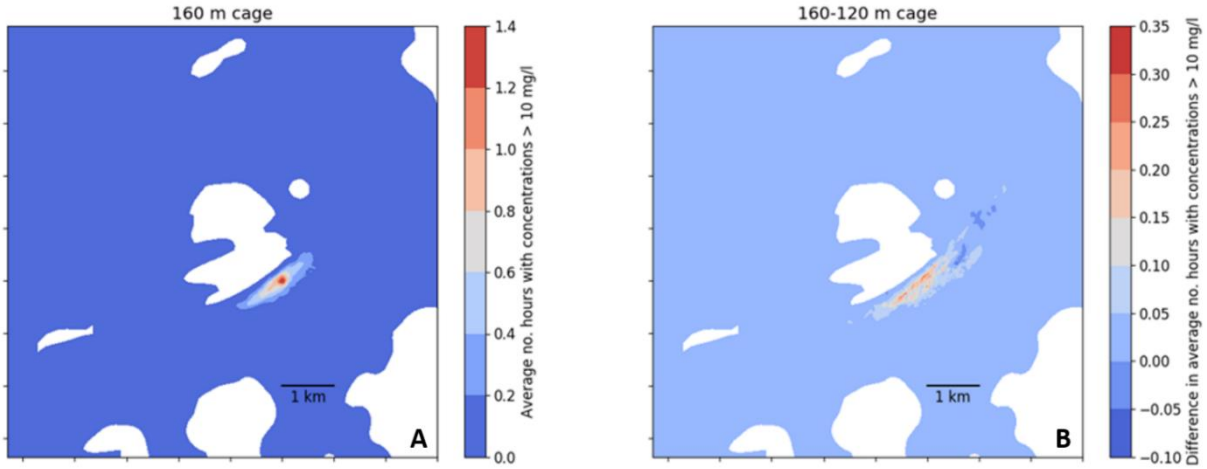

Figure 43. Average number of hours that a release at Indre Skjervøy results in concentrations above 10 mg/L after release from a 160 m cage (A), and the difference between 160- and 120 m cage releases (B)

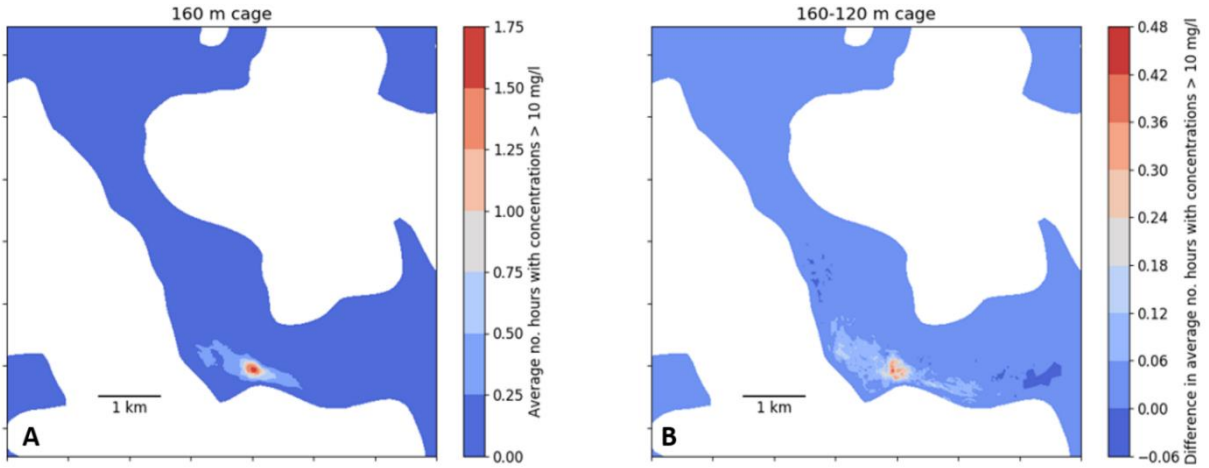

Figure 44. Same as Figure 43 for Austvika.

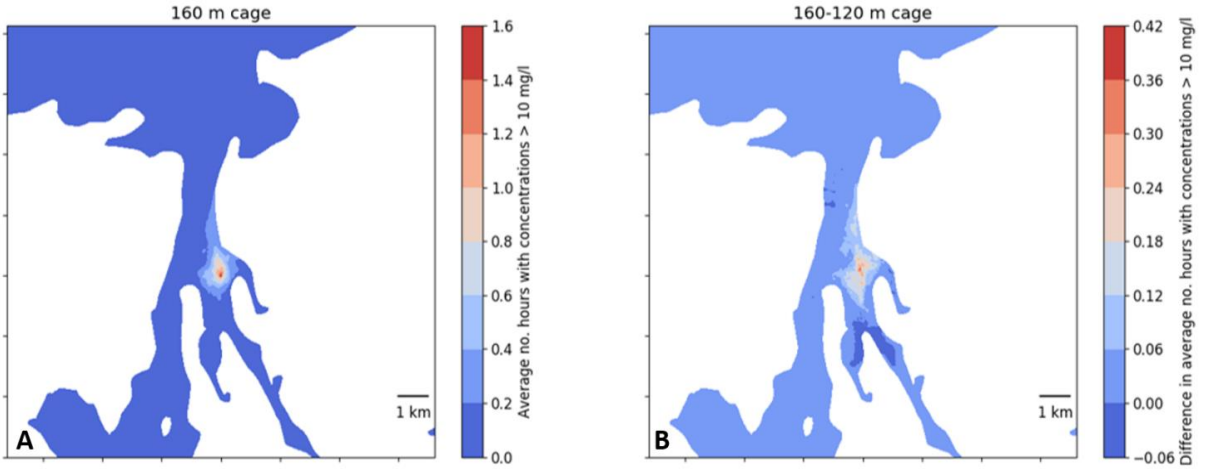

Figure 45. Same as Figure 43 for Kjelneset.

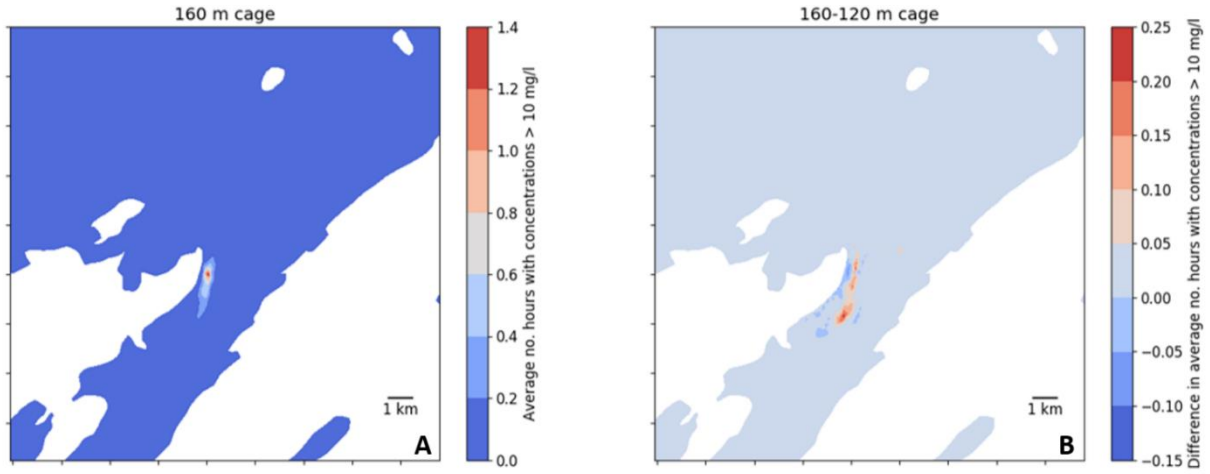

Figure 46. Same as Figure 43 for Jakobstensvika.

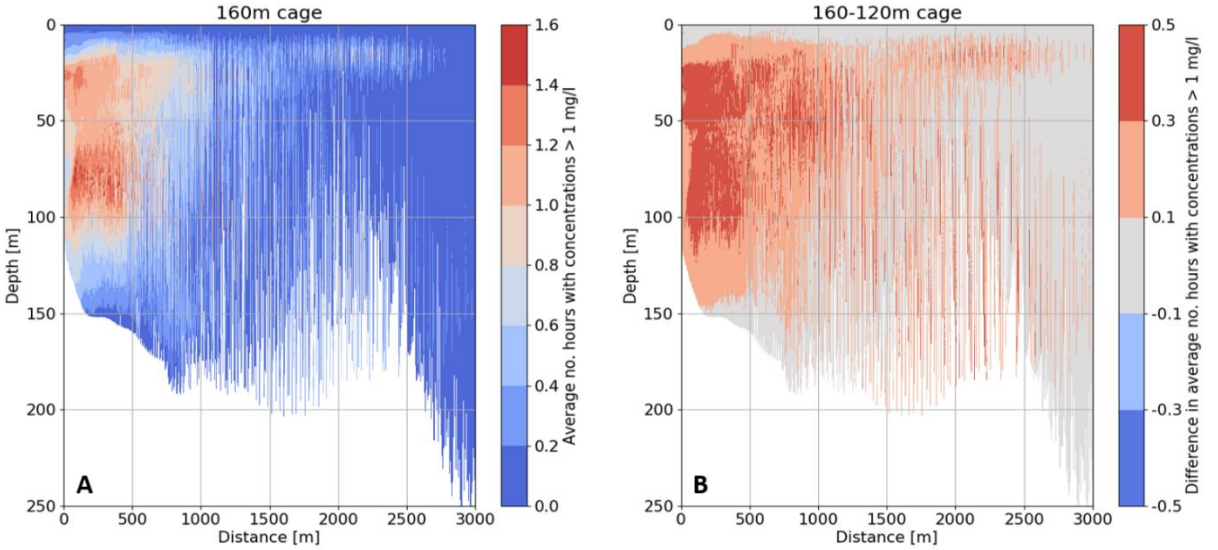

Figure 47. Average number of hours with concentrations above 1 mg/L after release from 160 m cage (A), and difference between 160 and 120 m cage (B) at Indre Skjervøy.

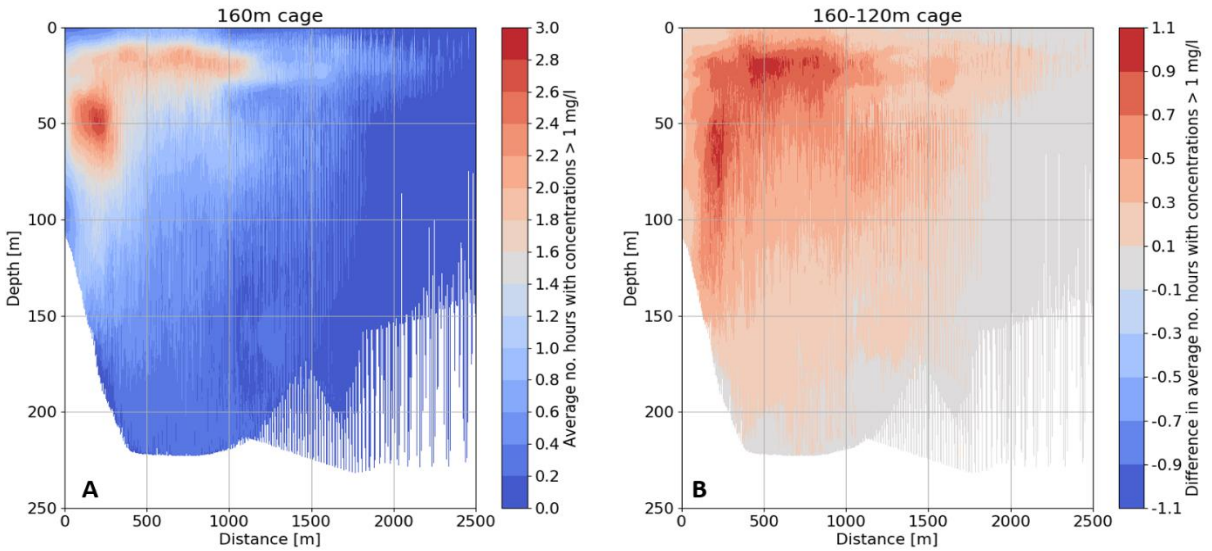

Figure 48. Same as Figure 47 for Austvika.

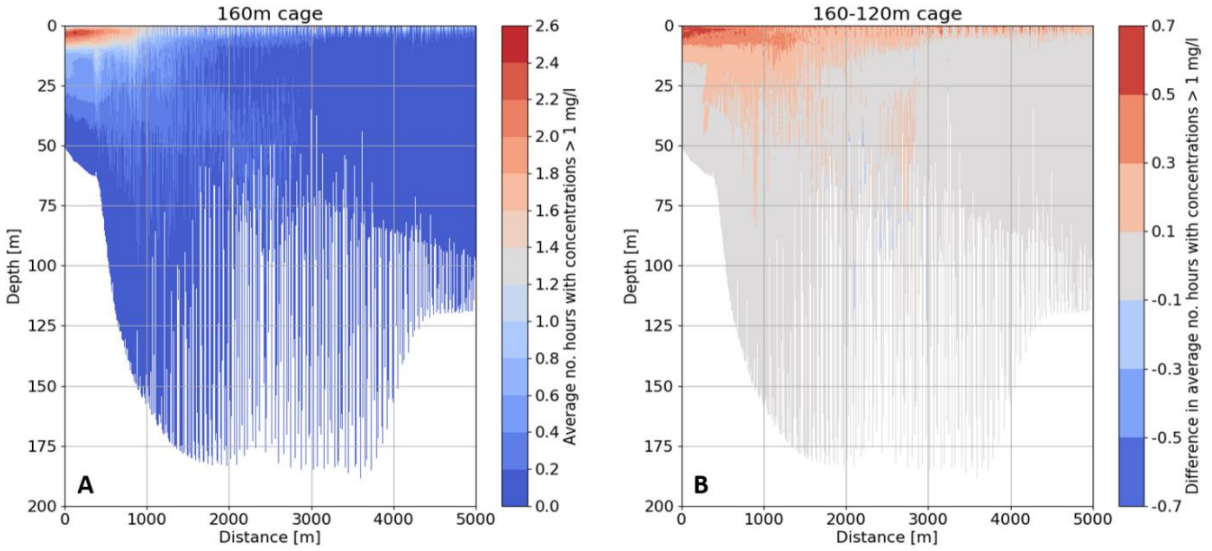

Figure 49. Same as Figure 47 for Kjelneset.

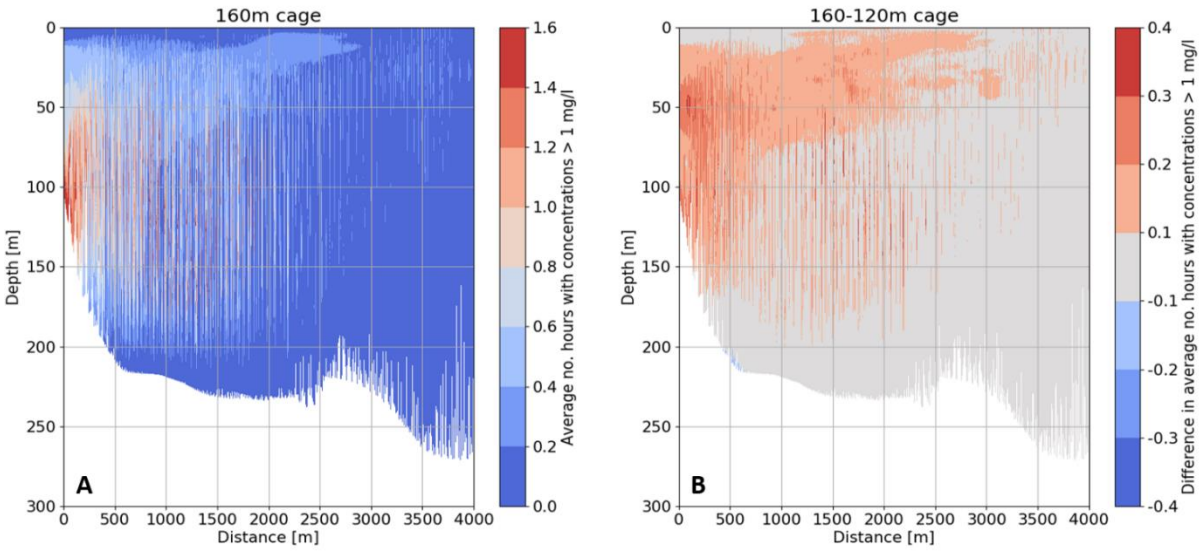

Figure 50. Same as Figure 47 for Jakobsteinsvika.

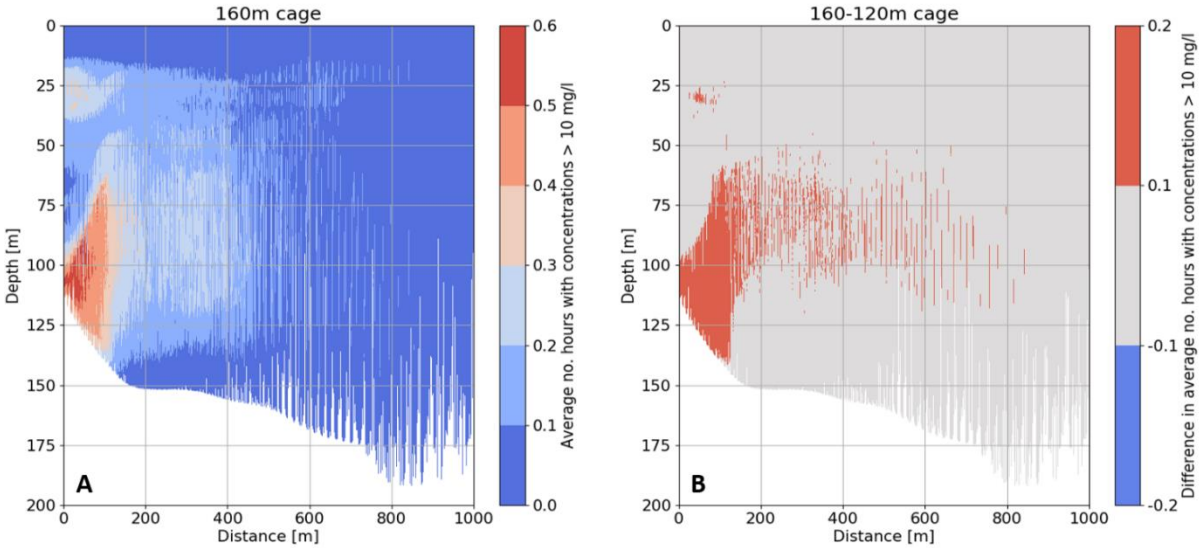

Figure 51. Average number of hours with concentrations above 10 mg/L after release from 160 m cage (A), and difference between 160 and 120 m cage (B) at Indre Skjervøy.

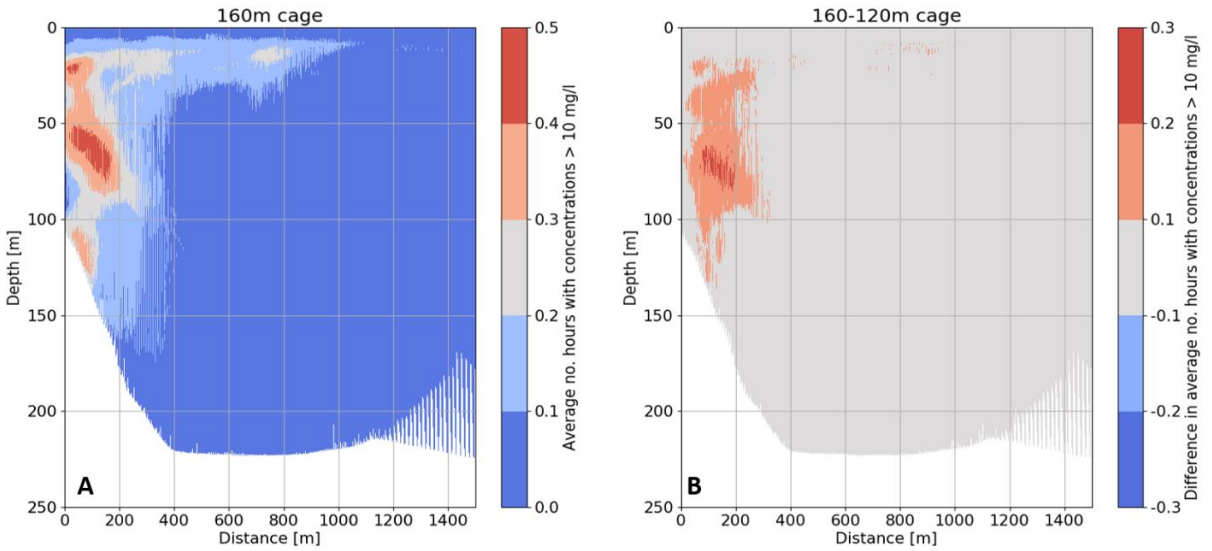

Figure 52. Same as Figure 51 for Austvika.

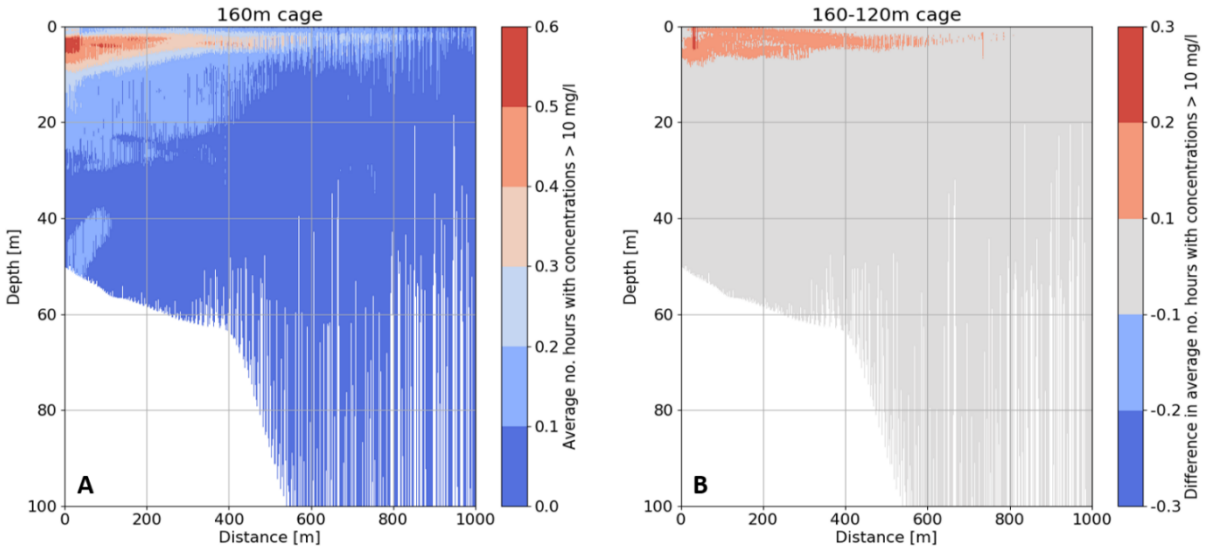

Figure 53. Same as Figure 51 for Kjelneset.

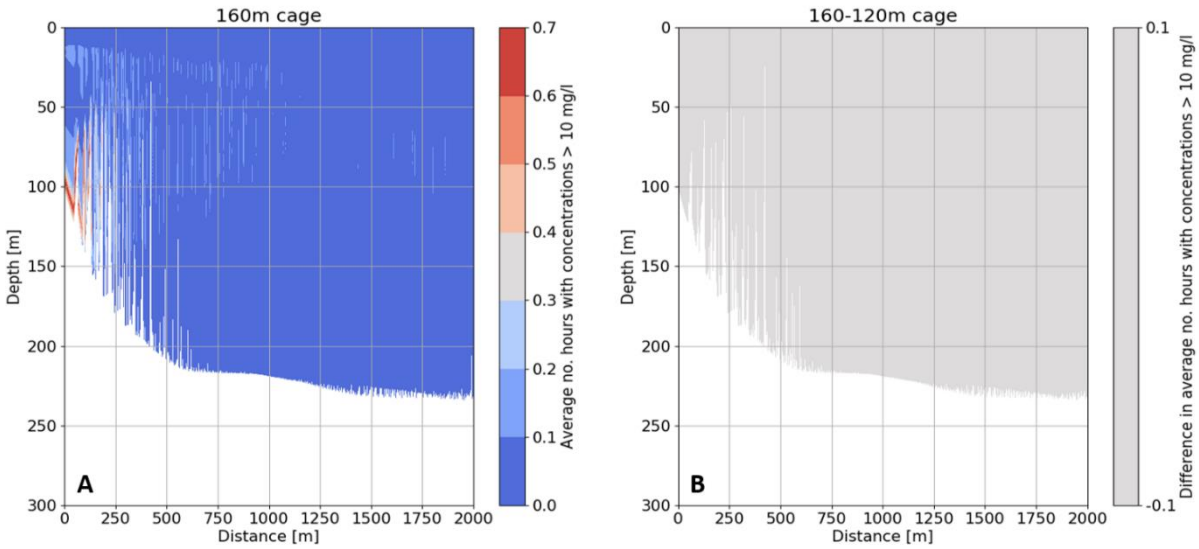

Figure 54. Same as Figure 51 for Jakobsteinsvika (panel B indicate no difference between the two releases).

6.6.3 Multi-cage releases

From the single cage releases, it is observable that the average number of hours with concentrations above 1 mg/L after a single release is above 6 hours for Austvika (Figure 40). Since the minimum amount of time between releases in the 4 cage experiments is 6 hours, the effect of the previous release on the next is on average little, but for low concentrations it can still make a difference. Maximum concentrations at all 4 locations for the 4-cage release is shown in Figure 55-Figure 58 in the left panels. In the right panels the difference in maximum concentration between the 4-cage release and the single case releases are shown (4-cage – single release 120 m). Since the releases in the 4-cage delousing operations is from 4 different cages, while the single releases are always from the same cage, the maximum concentrations close to the farm are generally higher for the former case. However, at some distance from the fish farm the maximum concentrations from the 4-cage releases is not necessarily higher, and especially far from the fish farm the single releases have a higher maximum concentration. This is probably due to the 48 single case releases being more evenly spread throughout the year, with higher variability in weather and current conditions.

The average number of hours with concentrations above 1 and 10 mg/L after a 4-cage delousing simulation are shown in Figure 59 and Figure 60, respectively. In this case Kjelneset and Austvika have concentrations above 1 mg/L for more than 10 hours on average, while the more exposed locations also here have somewhat lower exposure times. For concentrations at or above 10 mg/L the average exposure time is highest at Indre Skjervøy with up to 3.5 hours.

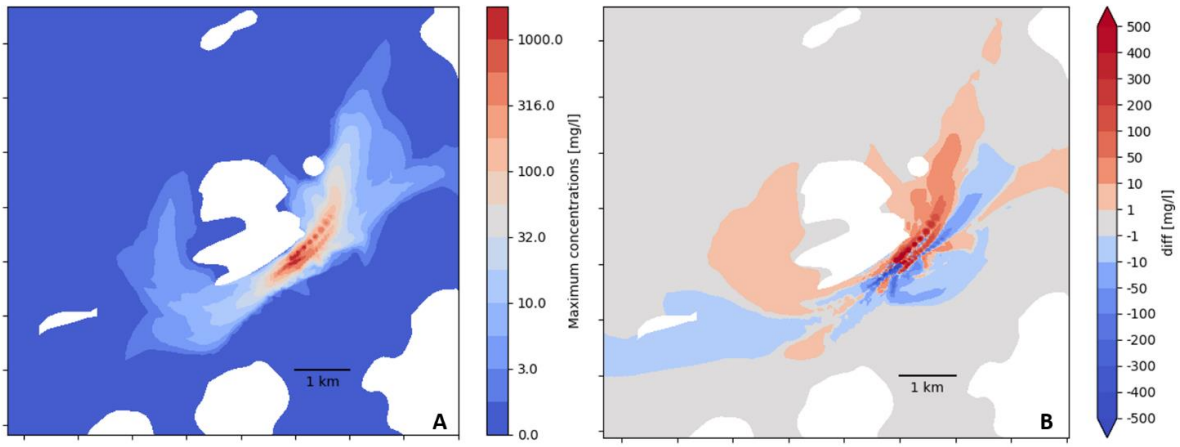

Figure 55. Maximum concentrations at Indre Skjervøy during 12 simulated farm delousing operation (4 consecutive releases from different cages for each operation) (A) compared to 120 m single cage release (B).

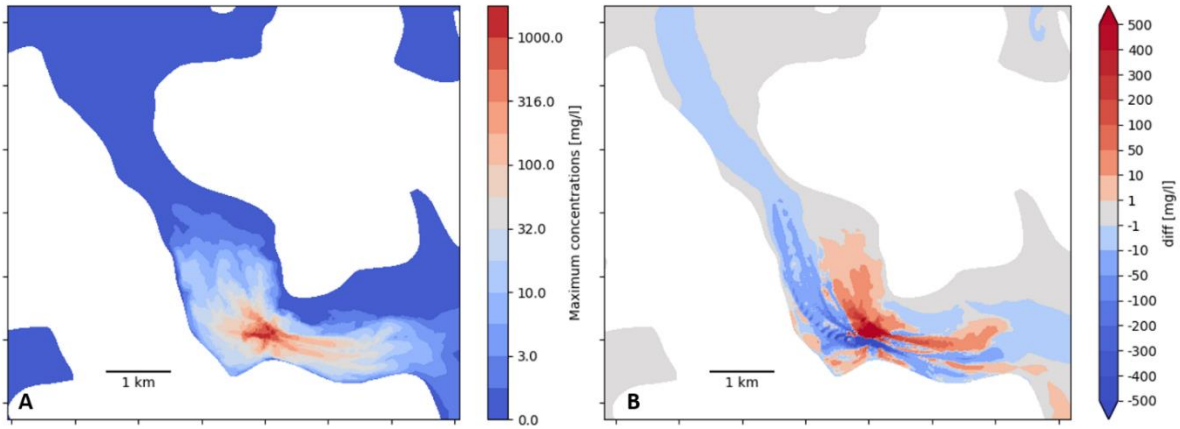

Figure 56. Same as Figure 55 for Austvika.

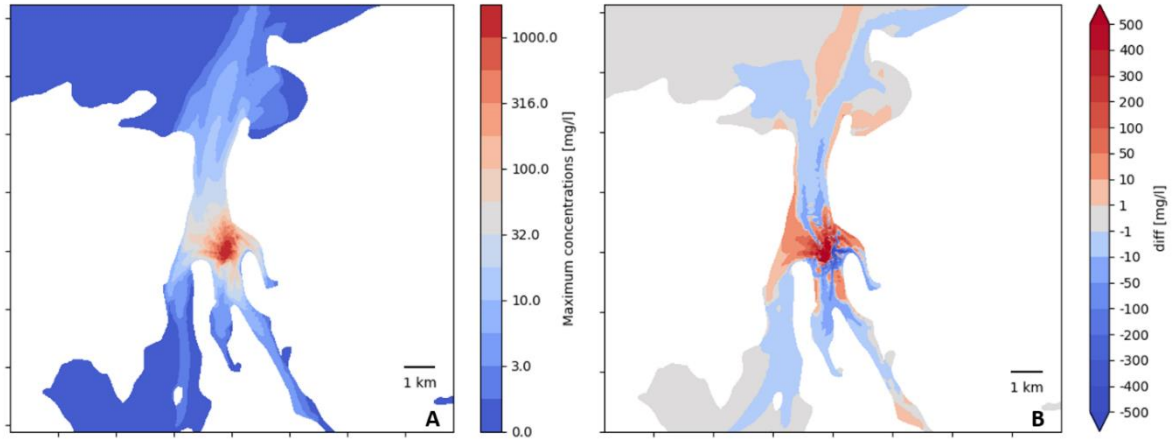

Figure 57. Same as Figure 55 for Kjelneset.

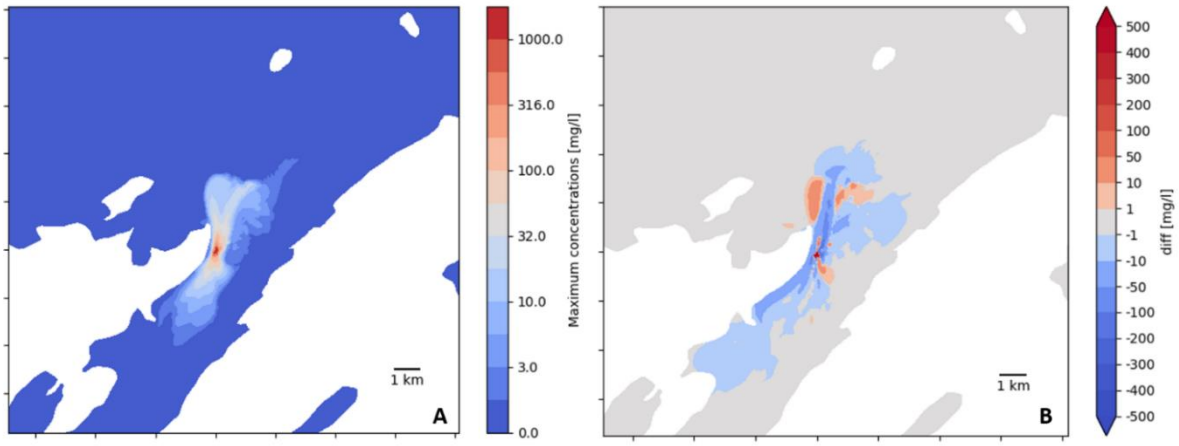

Figure 58. Same as Figure 55 for Jakobsteinsvika.

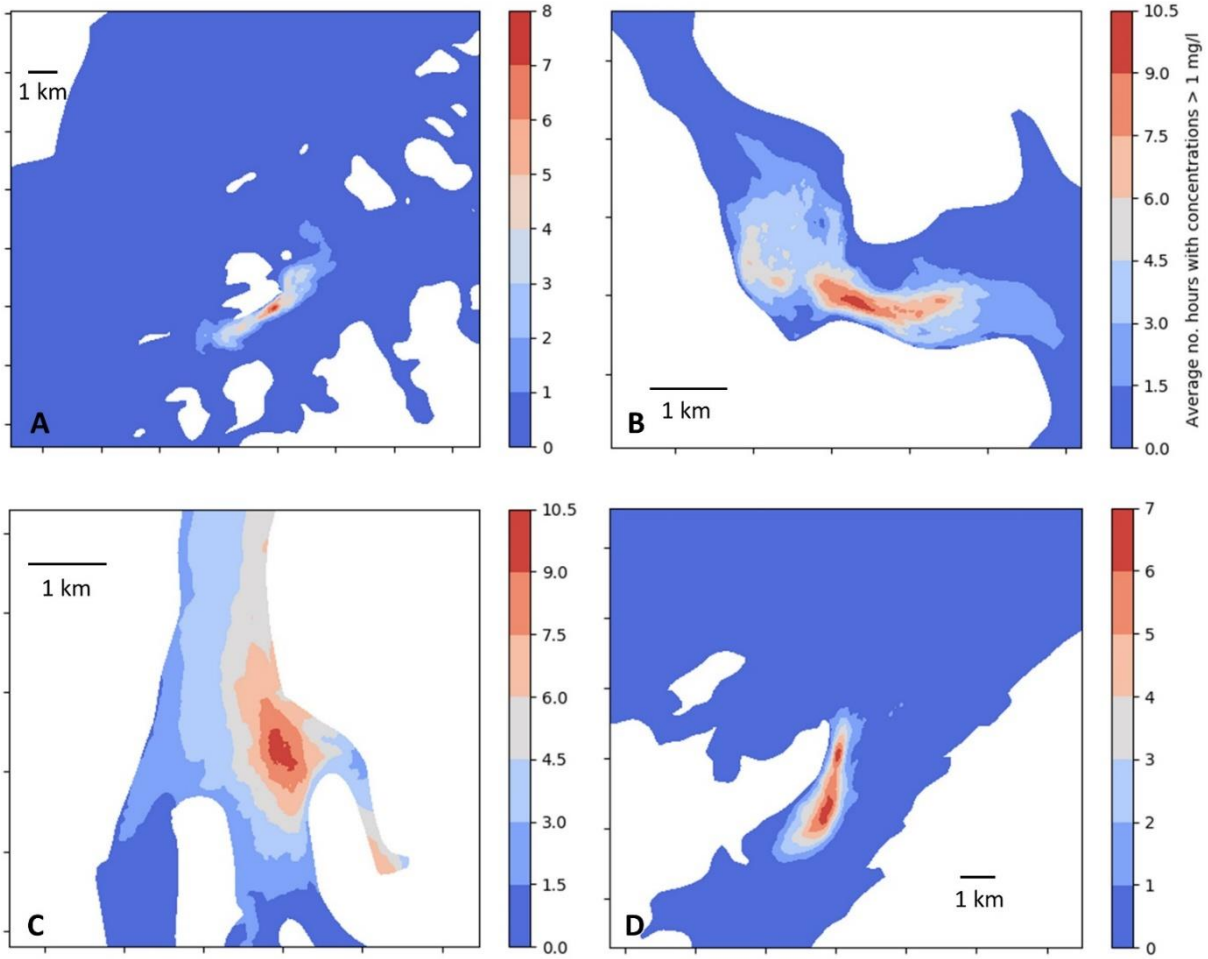

Figure 59. Average hours with concentrations above 1 mg/L for 4-cage delousing. Locations are Indre Skjervøy (A), Austvika (B), Kjelneset (C) and Jakobsteinsvika (D).

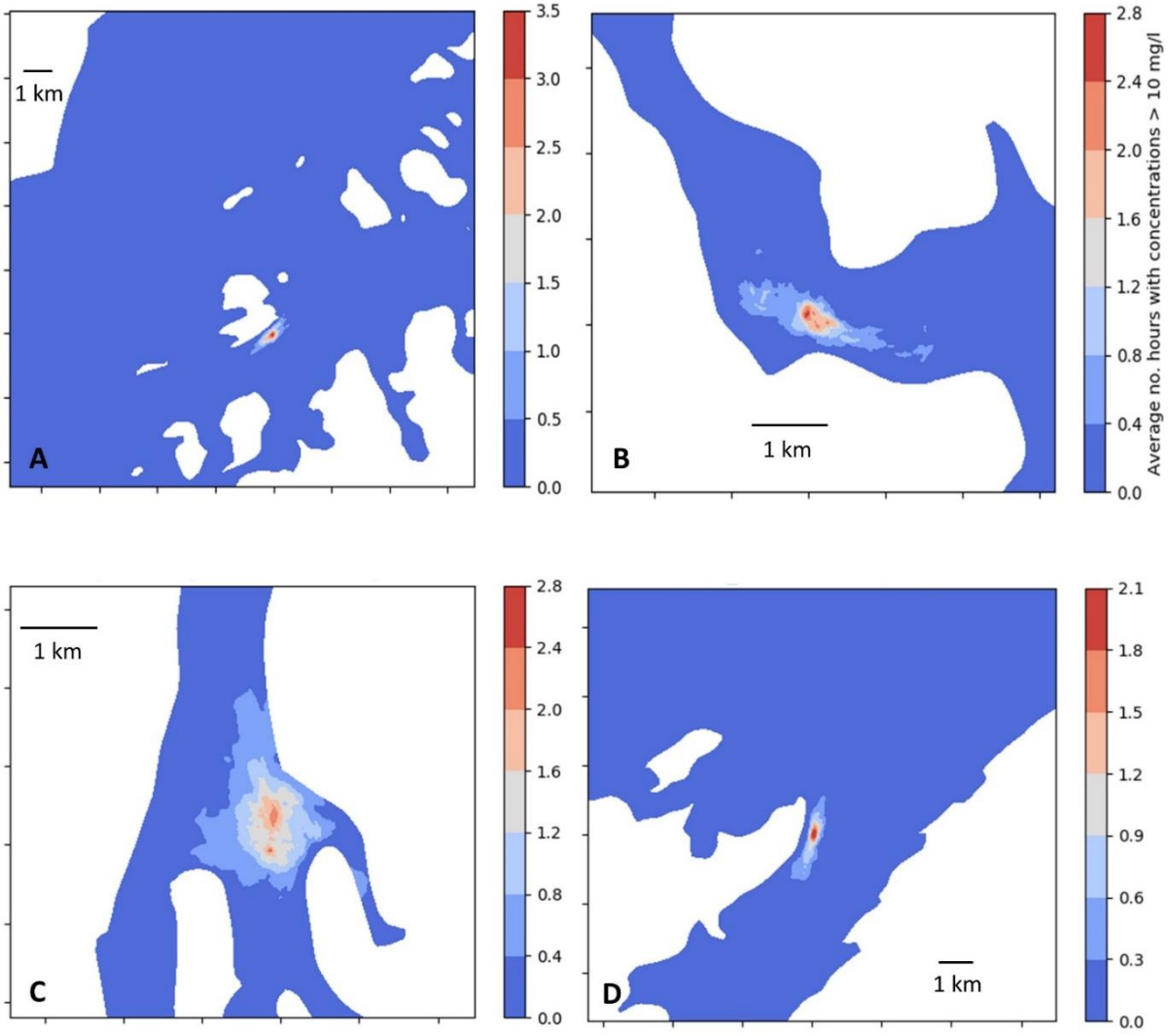

Figure 60. Average hours with concentrations above 10 mg/L for 4-cage delousing. Locations are Indre Skjervøy (A), Austvika (B), Kjelneset (C) and Jakobsteinsvika (D).

6.6.4 Comparison of spreading from wellboats and cages

To simulate the spreading from wellboat the volume used is 3 000 m^3. In order to compare this with a delousing operation in cages, an extra 4-cage spreading scenario was run for Jakobsteinsvika, where the mean value reported from Aqua Pharma was utilized (7 700 m^3). Although the volumes are not the same, they are comparable, and represent realistic volumes for the respective cases. As illustrated in Figure 61, there is a big difference in maximum concentrations reached when comparing release after delousing in the cages with the wellboat. Clearly, the delousing in a wellboat and subsequent release of H_2O_2 yields far lower concentrations in the water masses at this location. Also, the average hours with concentrations above 1 mg/L is much lower for the wellboat release as shown in Figure 62. Furthermore, the concentrations are so low (Figure 61) that it was not possible to plot any time with concentrations above 10 mg/L (like Figure 60). The results here indicate that the impacts from wellboat releases are considerably lower than the impact of direct cage releases.

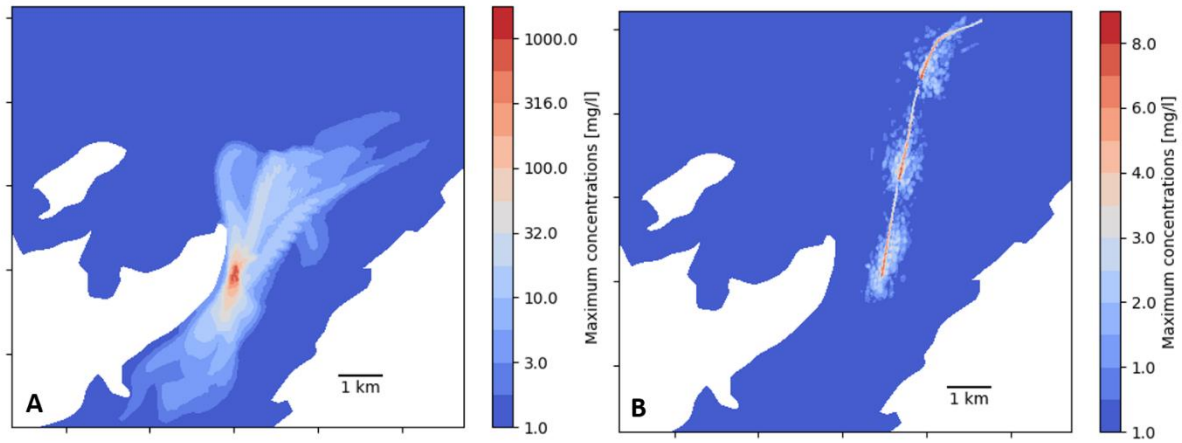

Figure 61. Maximum concentration from all simulations from 4 cage releases at Jakobsteinsvika (A) and wellboat (B). ***Note different scales on the y axis****.*

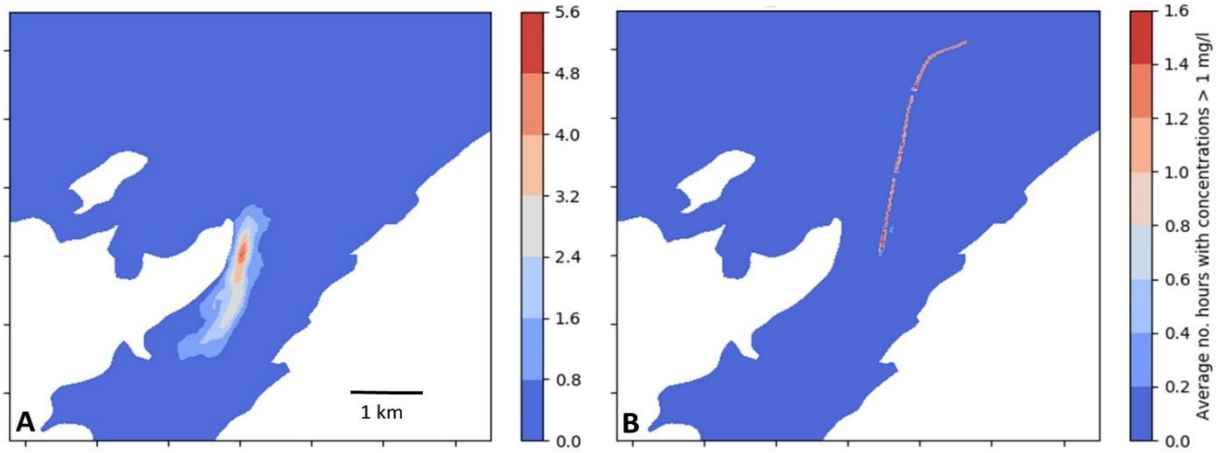

Figure 62. Average hours with concentrations above 1 mg/l for 4-cage delousing (A) and Wellboat (B).

Note different scales on the y-axis.

6.7 Discussion and conclusion

Since the density of H_2O_2 is higher than sea water, the mixture used for delousing is heavier than the ambient water and may sink to deeper layers. For the cage-release experiments the initial sinking process is calculated using existing theory for rising/sinking of thermals, providing an initial state for the hydrostatic ocean model. For the wellboat releases, a non-hydrostatic model is used for the initial sinking process. Studying the potential sinking at the 4 locations presented above demonstrates how sensitive the sinking depth and initial dilution is to the stratification. For the three locations Indre Skjervøy, Austvika and Jakobsteinsvik the sinking depth follows the seasonal pattern of the stratification, with deeper sinking in the colder months. However, for the highly freshwater influenced location Kjelneset there is a more permanent stratification and hence the sinking depth is shallow year-round. Since the sinking process itself leads to a dilution of the H_2O_2 mixture through entrainment of ambient water, the difference in sinking depth has an effect on the initial concentration. Furthermore, the sinking depth may also affect the horizontal spreading since there is often a vertical gradient in the current and the surface-near current is more affected by the wind.

From the single cage releases, we find that for all four locations concentrations up to about 300 mg/L can occur within about 1 km from the release sites. Concentrations of 10 mg/L are found within about 5 km from the release sites. Comparing the releases from 160 m cages with that from 120 m cages, it can be seen the concentrations are up to 500 mg/L higher in the immediate vicinity of the release site. Differences in concentration between 10 – 50 mg/L occur up to four kilometers away. The modelled concentration of H_2O_2 decays quite rapidly from the initial concentration due to mixing, and concentrations above 10 mg/L can be found on average between 1.4 and 1.75 hours after the spill (160 m cages). For concentrations above 1 mg/l the potential exposure time is longer (between 3.2 and 6.4 hours), and the difference between the locations is greater. For this low limit, the more sheltered locations Austvika and Kjelneset have longer exposure times than the locations further out towards the open ocean (Indre Skjervøy and Jakobsteinsvika). Only at Kjelneset, which is most affected by fresh water from rivers, does the highest exposure times occur close to the surface. For the other locations, concentration values exceed the 1 and 10 mg/L limits longer at depths between 25 -100 m depth. This further demonstrates how important the stratification is for the spreading of H_2O_2.

The multi-cage releases were set up to mimic a realistic delousing operation at a fish farm, releasing from one cage at the time from 4 cages during a span of 2 days. Since the minimum time between releases is 6 hours, the effect of one spill on the next is limited in terms of maximum concentrations. With four releases, the total exposure time is naturally higher than for the single releases.

In the wellboat experiments, the initial concentrations are far lower than in the cage releases since the wellboat mixes in ambient water into the tanks and releases the H_2O_2 mixture while in motion. In order to compare delousing in cages with delousing in wellboats, the 4-cage experiment was run an extra time at Jakobsteinsvika, with a release volume that corresponds to the average volume reported by Aqua Pharma for 120 m cages (7 700 m^3). While this is still higher than the volume released by the wellboat, the comparison is between two realistic delousing operations for the same farm using the two methods. Comparing the results from the two cases, it can be seen that the maximum concentrations from the cage-releases exceeds the maximum concentrations from the wellboat releases by more than a factor of 100. The difference in exposure time for concentrations over 1 mg/L is not as large, but still this retention time from the cage releases exceeds the wellboat releases by a factor of 3.5. These results indicate that delousing by H_2O_2 from a wellboat poses a lower environmental risk than delousing in the cages.

It should be noted that the degradation of H_2O_2 is omitted in the model, and hence the only factor leading to lower concentrations in time in the model is the dilution due to mixing with the ambient water. For high concentrations and short periods in time, the mixing process greatly dominates the natural degradation, but for low concentrations persisting for several hours, the degradation could be important. Here we have considered concentrations above 1 mg/l, which for the single case releases on average persists up to 6.4 hours (Austvika), and within this time scale mixing is the dominating effect.

7 Risk assessment

7.1 Background and metrics used in risk assessment

The definitions of risk can differ among risk assessment methodologies, but the basics of risk assessment related to the aquatic environment are universal. The risk is assessed by comparing the exposure of (a part of) the ecosystem to a chemical with the sensitivity of the ecosystem for this chemical. The exposure is usually represented by the PEC, as in the present study, while the sensitivity is expressed in a PNEC. A PEC:PNEC ratio higher than 1 indicates that unacceptable effects on organisms are likely to occur; the higher the ratio, the more likely that unacceptable effects may occur. According to international guidelines, risk reducing measures should be taken/discharges should be decreased when a PEC:PNEC ratio is higher than 1. For delousing substances, it is obvious that relatively high concentrations, far above PNEC, will occur close to the release site. If delousing chemicals are released into the environment PEC will exceed PNEC in water volumes of various sizes, as described in the previous chapter. If this practice continuous the question that environmental managers must answer is within which areas concentrations above PNEC can be accepted?

As previously described PNECs can be derived by applying assessment factors to toxicity data obtained for single species if data are scarce. In this case data for the most sensitive species and the most sensitive endpoint should be used. Species sensitivity distributions (SSDs) are used in ecological risk assessment for extrapolation of the results of toxicity tests with single species to a toxicity threshold considered to be protective of ecosystem structure and functioning. With the PNEC being the 5 percentile of a SSD based on LC_{50}, the PEC:PNEC-ratio together with the slope of the SSD give a quantification of the likelihood (probability estimate) and a characteristic of the extent of effects (fraction of species being affected by the toxicant) (Figure 63).

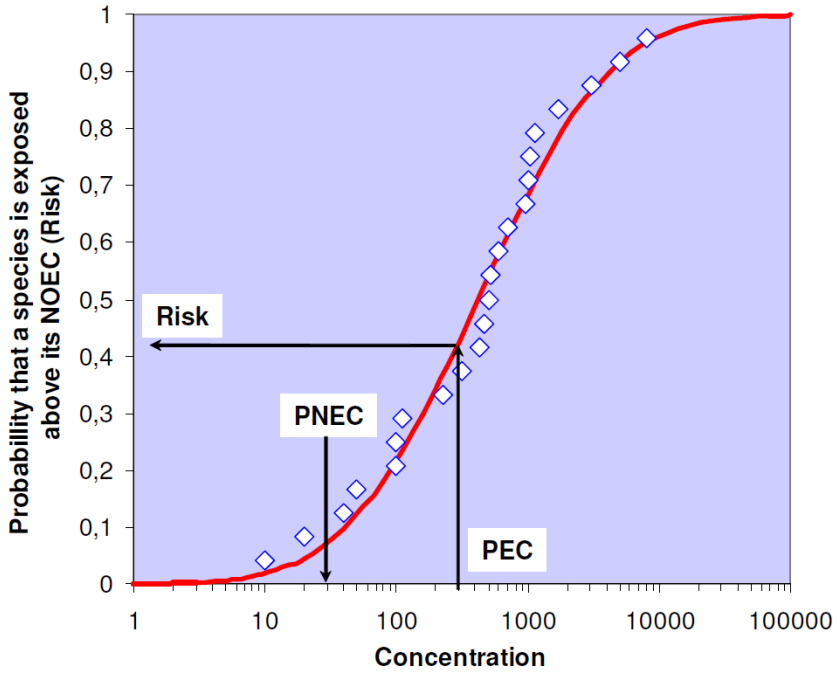

Figure 63. Use of SSD for translating PEC values to risk values (from Smit et al. 2005).

In many cases risk assessments must be based on short-term exposure data, i.e. from laboratory studies. In cases where the exposure to a compound/a chemical mixture is constant, i.e. if emissions are constant, this may lead to an under-estimation of risk. However, for emissions of delousing chemicals short-term exposures will give an adequate and realistic picture of risk as these are released in short lasting pulses. However, in many cases several pulses can follow each other at varying time intervals, e.g. when several cages are de-loused in a sequence (several plumes in a short time period), or when de-lousing must be performed at intervals (e.g. once or twice a year).

As previously discussed, the advantage of using NEC in ecotoxicology is that sensitivity among species can be compared regardless of how long the different species are exposed to a chemical. The tests performed in the current project and by Refseth et al. (2016), and data from the literature, reveal that there is a large variation in sensitivity towards H_2O_2 among species. Possible reasons for differences are discussed in the ecotoxicology result section below. The results show that the time for effects (mortality) to occur vary between species. This means that if an organism is exposed to a transient, short-lasting pulse with concentrations above NEC, no effects may be recorded. Thus, environmental risk can be overestimated if we only consider the relationship between PEC and PNEC. Model results from this study show that concentrations above the effect concentrations occur in the environment. The question is whether the concentrations are present long enough for the animals to die. It is therefore important to assess the durations of the exposure to different concentrations, and this has been done in the dispersal modelling.

In Refseth et al. (2016) calculations around the time aspect of NEC was performed by using the DEB model. The time it takes for the different species to reach effect (mortality) at different H_2O_2 concentrations was calculated (when possible, Table 11). The method is described in Baas et al. (2010). The results reveal that there is a great difference in how long the different species need to be exposed to a given concentration in order to achieve effect. For those species that respond rapidly to H_2O_2, there may be concentrations present in the environment long enough for mortality to occur, such as for deep-water shrimps. For other species, such as lumpfish and cod eggs, H_2O_2 will probably not be long enough in the environment for survival to be affected (Refseth et al. 2016).

Table 11. Time needed to reach effect (hours) for C. lumpus, P. borealis, P. flexuosus, P. elegans and G. morhua eggs (from Refseth et al. 2016).

	t (h) ***50 mg/l***	***t (h)*** ***100 mg/l***	***t (h)*** ***150 mg/l***	***t (h)*** ***200 mg/l***	***t (h)*** ***250 mg/l***	***t (h)*** ***300 mg/l***
Cyclopterus lumpus	-	-	16.0	8.5	6.0	4.6
Pandalus borealis	3.2	1.4	0.9	0.6	0.5	0.4
Praunus flexuosus	-	30.1	11.7	7.6	5.7	4.5
Palaemon elegans	-	15.3	8.5	5.9	4.6	3.7
Gadus morhua egg	-	-	81.5	27.7	18.5	14.0

However, one should notice that data from this table is generated without studying delayed effects. New published data from Bechmann et al. (2019) revealed that mortality occurred at concentrations and exposure time lower than those defined in this table for *P. borealis.* Delayed effects and differences between ecotoxicological results are described more in detail in chapter 7.2.

The following chapters first assess the risk for local ecosystems based on the HC_5-value and PNEC calculated from the SSD-curve and modelled concentrations and exposure times. Then, the risk for the selected species that are key species in the ecosystem or that has a commercial value was assessed to illustrate the large differences that occur between species. Several factors may explain differences in sensitivity between species, such as life stage, size, nutritional status, temperature etc. Also, variation in sensitivity within the same species are often reported in ecotoxicological studies. When risk is assessed for a species, it is important to compare all the available ecotoxicological data for the species in question, as the ecotoxicological metrics used in risk assessment such as LC_{50}, EC_{50} and NEC can vary from one study to another for the same species. Within the SSD curve such variations are considered by the assessment factor.

7.2 Acute versus chronic and delayed effects

The endpoint used in the ecotoxicological experiments performed within this project and in the previous project by Refseth et al. (2016) is mortality. For some species the mortality endpoint may be appropriate to use. Hansen et al. (2017) studied oxidative stress in *C. finmarchicus* exposed to H_2O_2 concentrations close to the LC_{50}-value. They found that H_2O_2 did not significantly affect the antioxidant system in this species. They suggest that aqueous H_2O_2 exposure do not cause cellular accumulation with associated oxidative stress, but rather produced acute effects on the copepod surface (carapace). For this species mortality may therefore be a suitable endpoint.

However, for many species sub-lethal effects may occur at concentrations below acute limits (Barata et al. 2002), and recently increased attention has been given to the inclusion of sublethal effects in risk assessment. Risk assessment of chemicals used to combat pest species without considering sublethal effects has failed to protect the terrestrial environment due to sensitivity of some species (Sanches-Bayo & Tennekes 2017). To avoid underestimation of risk, a new framework is proposed that combines the mandatory introduction of new toxicity endpoints. Chronic toxicity tests should be a requirement for assessing delayed mortality as well as sub-lethal population endpoints that are crucial for the survival of species (Sanches-Bayo & Tennekes 2017). In a recent study, it was concluded that acute toxicity testing is not sufficient to evaluate effects of some of the chemicals used to combat sea lice (Lillicrap et al. 2015). In the current project an assessment factor has been added to the SSD curve to cover potential sub-lethal effects, other than those of classic acute toxicity tests. Hence, potential sub-lethal effects are taken into account in the overall PNEC value. However, sub-lethal effects are not considered in the ecotoxiclogical metrics for the individual species, as no assessment factor have been added to the LC_{50}, NEC and EC_{50} values. Thus, when risk is assessed for some individual species (chapter 7.4), underestimation of risk may occur since sub-lethal effects are not accounted for.

Sub-lethal effects of H_2O_2 has been documented in several different species. E.g. one study on adult and nauplii of different copepods species showed sub-lethal effects, such as changing feeding behavior, after exposure to 5 mg/L H_2O_2 at 7 °C. A concentration of 10 mg/L and an exposure time of 10 minutes resulted in total paralysis of the copepods (Van Geest et al. 2014). Other sub-lethal effects, occurring at concentration well below the treatment concentrations and under LC_{50} values, have also been documented. Sub-lethal effects reported for H_2O_2 are altered swimming activity, changes in heart rate, behavioral changes, decrease in the total ratio of glutathione/oxidized glutathione, oxidative stress, branchial DNA damage, increase in magnesium superoxidase dismutase, rapid increase in the expression of glutathione peroxidase in the hepatopancreas, hemocytes and gills (Veeramani & Baskharhungam 2011, Fu et al. 2013, Pellegri et al. 2014, Van Geest et al. 2014;,Wang et al. 2014, Bownik & Stepniewska 2015). Brachial DNA damage was documented in *Sinopotamon henanense* at concentrations of 0.17

mg/L (Pellegri et al. 2014). Furthermore, recent studies in Norway have documented sub-lethal effects after H_2O_2 exposure, that can later affect survival (Escobar Lux 2016, Fagereng 2016, Bechmann et al. 2019, Fang et al. 2018, Haugland et al. 2019). The Norwegian studies that documented delayed effects of H_2O_2 on different species demonstrates the importance of following the animals after the termination of the experiments, to look for possible post-exposure effects. Underestimation of sensitivity of organism may occur if the ecotoxicological metrics used in risk assessment (e.g. LC_{50}, NEC) are estimated without including potential sub-lethal/delayed effects.

Haugland et al. (2019) studied impacts of H_2O_2 on *S. lattissima* and concluded that it was essential to keep the kelp in the laboratory for at least 7 days after terminating the experiment to be able to determine mortality with certainty. In a study of Fang et al. (2018), where two polychaeta species were reintroduced into clean sea water after termination of an exposure experiment (1-hour exposure), both species experienced high cumulative mortality during a 72 h post-exposure period, revealing delayed effects. Furthermore, in the study of Escobar Lux (in Refseth et al. 2016) delayed effect were observed on *C. finmarchicus*. After the termination of the experiment (1-hour exposure to H_2O_2), the surviving *C. finmarchicus* were placed in clean water, and a 100 % mortality was observed 24 hours after the end of the experiment. In an experiment with the shrimp (*Palaemon elegans*) and the mysid (*Praunus flexuosus*) exposed to H_2O_2 delayed effect were studied (Brokke 2015). The shrimp were followed 12 hours after the end of the experiment. Mortality was observed on the shrimp after they were moved to clean water, revealing delayed effects. Finally, in the recent published study Bechmann et al. (2019) documented mortality of deep-water shrimp (*P. borealis)* 2-4 days after the first 2 h puls of exposure. If Bechmanns experiments had been terminated after 24 h, no effects would have been discovered, since shrimp appeared to be normal and no mortality was observed. In the study performed by Refseth et al. (2016) on the same species, but with higher exposure concentrations, the experiment was terminated after 24 h. Hence the shrimp were not followed in clean water after the end of the experiments. Therefore, potential delayed effects were not included in that study. When the NEC and LC_{50} values, as well as "time needed to reach mortality" from this study are used in risk assessment, risk may be underestimated. NEC implies that no effects occur regardless of how long the animals would be exposed to the chemical. Mortality of deep-water shrimps have been demonstrated at concentrations below the no effect concentration of 23 mg/L established in Refseth et al. (2016). Mortality was observed after 2 h exposure at concentrations of 15 mg/L. After 2 h exposure to 1.5 mg/L on 3 consecutive days, mortality was also observed (Bechman et al. 2019). This shows that mortality of deep-water shrimp may occur at levels which are below the NEC value and also the "time to reach an effect" calculated in Refseth et al. (2016).

A later experiment performed by APN on deep water shrimps, at similar concentrations and exposure time as used by Bechmann et al. (2019), but lower than Refseth et al. (2016), delayed effects were not observed even though the shrimp were observed for four weeks after exposure (Frantzen et al. 2019). The study was conducted at slightly lower temperatures compared to Bechmann (2019). One possible explanation for higher sensitivity of shrimps in the study of Bechmann et al. (2019) could be related to temperature, as H_2O_2 has been shown to be more toxic at higher temperatures. E.g. in the study of Rach et al. (1997), toxicity of H_2O_2 increased for all species tested as water temperature increased. It is recommended that H_2O_2 should not be used to delouse salmon at temperatures above 13°C, due to the toxicity to the fish at warmer temperatures (Terapiveileder 2012). A slightly higher temperature was used in the study of Bechman et al. (2019) because the *in situ* temperature for the shrimp population was higher in western Norway compared to northern Norway where the shrimps from Refseth et al. (2016) were collected. However, as the temperature difference between the studies of Bechmann et al.

(2019) and Frantzen et al. (2019) was small, it is likely that other factors, such as e.g. different sizes, life stages (moulting stage) can be important.

The differences described above illustrates the importance of assessing and comparing different ecotoxicological results available for the same species in risk assessment procedures. Generally, when there are more data available, the most sensitive measures/endpoints should be chosen, or the one study with the highest quality.

7.3 Risk for the communities

The ecotoxicological metrics derived from the SSD curve are used in characterizing the status of the environment and may be used by policy makers, regulating production, use, and emissions of chemicals. HC_5 is set to the value considered to protect 95 % of the species in the ecosystem. Based on the SSD-curve the HC_5 is 0.70 mg H_2O_2/L. Algae have shown to be very sensitive and are the main reason for this low HC_5. However, the crustaceans were only slightly more robust than the algae. For most tested species in the current project, the NEC and LC_{50} values are higher than the HC_5 value. However, based on the principles of SSD distribution, the more species you test, the more data you will probably have both below and above and around this value, distributed along the SSD line (see principles of SSD distribution, Figure 11). Hence, chances are that in the ecosystem, there will be more species with sensitivities between the HC_5 value and LC_{50} and NEC calculated in the current project.

Quantitative risk assessment can be done based on the PEC/PNEC comparison. The use of assessment factors will lead to a PNEC that can be compared to the PEC and will result in a risk characterization ratio (RCR), which is quantitative. The uncertainty of this approach will be high when data for individual species are used. This is compensated by the use of higher assessment factors. For SSD's this uncertainty is much lower and in the SSD calculated in the current project there are 34 species covering 7 phyla, hence the uncertainties are small. By comparing the PNEC (0.14 mg/l) to the PECs illustrated in the different maps in chapter 7, the risk can be quantified. The result from PEC/PNEC comparison reveal that risk ratio is relatively high within a relatively large distance from the release sites, indicating that there may be a relatively high chance for negative environmental effects to occur. The risk will however vary between geographical sites.

Marine algae form the basis for the food web, and it is important to protect primary producers in order to avoid cascading effects to other parts of the ecosystem. Protecting different community levels such as algae, species richness and diversity is essential for maintaining a healthy and well-balanced ecosystem (Clements & Rohr 2009). As shown in chapter 6.5 the physical environment on the different locations (currents, topography, stratification etc.) determine how H_2O_2 is distributed in the environment, and how rapidly it is diluted. Modelling results from the four selected test sites (Skjervøy, Kjelneset, Austvika and Jakobstensvika) are used to assess risk for the communities and different components of the ecosystem in the following text.

If PEC/PNEC ratio is higher than 1, negative environmental effects are expected to occur, and risk reducing measures should be considered or discharges reduced. Further analysis on the PNEC value can also be done, but with the PNEC derived from SSD, covering many species and phyla, we are almost at the end of refinements options for the PNEC value. The higher the PEC/PNEC ratio, the more likely it is that effects occur. In Chapter 6, concentrations in the environment after release of H_2O_2 were modelled. Figure 35 - Figure 38 in chapter 6.6.2 illustrates concentrations after release from 4 cages in the 4 test sites. To aid reading, Figure 36 showing the plot on concentrations from Austvika is copied under (from Chapter 6).

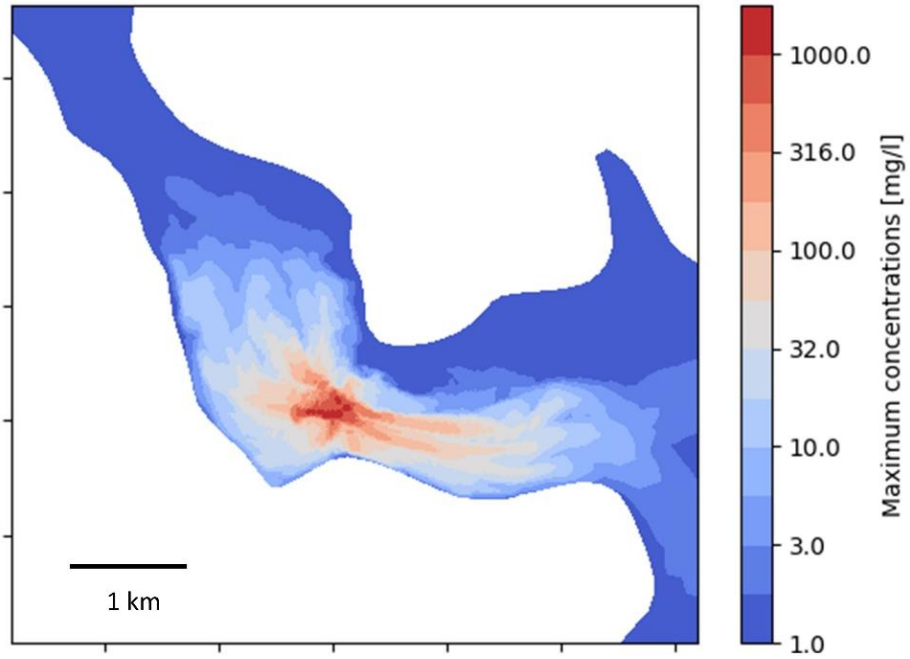

Figure 64. Maximum concentrations at Austvika during 12 simulated farm delousing operation (4 consecutive releases from different cages for each operation).

It must be stressed that 12 different scenarios are modelled and plotted in these figures, and the figures show the maximum concentration at each point over 12 different scenarios of 4-cage discharges. Thus, the plots show higher concentrations than from only one discharge from one cage, or from one discharge from 4 cages. The plots later in this chapter shows the amount of time the concentrations have been exceeding the PNEC, however, these plots do not show how much above the PNEC the concentrations are after release. For an organism, an exposure to a concentration close to the NEC or slightly above NEC, is obviously not the same as exposure to a concentration far above NEC. Hence, it is important to consider both the concentrations and the exposure time.

PEC concentrations are illustrated in chapter 6.6, and Figure 66 - Figure 69 illustrate release from 4 cages, 12 different scenarios. In all 4 test locations, H_2O_2 at different concentrations can potentially be found in relatively large areas, up to many kilometers away from the discharge point/points. Also, quite high concentrations (orange and red) can be found many kilometers away). When PEC values are compared to the ecotoxicological metrics listed in Table 6 - Table 8, for different species of algae, invertebrates and fish, respectively, results show that there are concentrations in the environment that are higher than all the ecotoxicological metrics for all the species studied, except for one intertidal species (*Gammarus sp*.), at all the test locations. The size of the areas where PEC is exceeding the ecotoxicological metrics depends on the model location and the sensitivity of the different species. The PEC/PNEC ratio is above 1 in all the colored areas of Figure 66 - Figure 69, indicating a risk for negative environmental effects to occur. Furthermore, Figure 64 shows that the PEC/PNEC ratio is high, especially in the orange and red areas, indicating a high risk for negative environmental effects to occur. The plot in Figure 64 show the maximum concentration from 12 scenarios combined. As pointed out earlier, the release from one discharge will cover a smaller area. The maximum concentration in this smaller area will, however, be similar.

To get a more detailed picture of areas where PEC is exceeding PNEC as well as the probability for this to occur, the plots in Figure 65 show probability for PEC exceeding PNEC for two

chosen model locations; Austvika and Jakobsteinsvika. It must be noted that these figures show plots from a 4-cage release scenario. The upper figures show probability for PEC exceeding PNEC both horizontally (left) and vertically (right). The figure reveals that the probability for PEC are exceeding PNEC is relatively high, both horizontally and vertically, in some areas and depths. The illustrations also reveal that there are differences in probability of negative effects depending on geographical locations. Note for instance that the influenced area cover the width of the fjord close to the release in Austvika, but only ~3/4 of width of the strait adjacent to the release in Jakobsteinsvika. See Chapter 6.4 for discussions on differences in currents and stratification for the different geographical test areas.

Only release from Jakobsteinsvika and Austvika are shown in Figure 65. Results from the other model domains reveal PEC exceeding PNEC there as well (the duration of PEC:PNEC >1 in these domains is shown later). When the release was performed using wellboat in Jakobsteinsvika, the PEC also exceeds the PNEC, but in a much smaller area /volume (results from wellboat will be discussed later). Since the illustrations reveal that PEC are exceeding PNEC, further analyses should be done according to risk assessment procedures. The illustrations above do not show how long animals can be exposed to harmful concentrations, and the probability for exposure above threshold values were not shown. Therefore, further analyses related to the time aspect were done for all model areas: Skjervøy, Austvika, Kjelneset and Jakobsteinsvika, and for releases from wellboat in Jakobssteinsvika.

Figure 66 to Figure 70 shows average time with H_2O_2 concentrations above PNEC for the four modelling areas: Skjervøy, Austvika, Kjelneset and Jakobsteinvika. Figure 65 show that there is a high probability that concentrations above PNEC can be present several kilometers away from the release site. There are however large variations between sites in expected exposure time and how large areas which are subjected to concentrations above PNEC (see Figure 64 - Figure 68). In Skjervøy, concentrations above PNEC can be found for up to ~27 hours (Figure 66), in Austvika, concentrations exceeding PNEC can persist in the water column for up to ~57 hours (Figure 67). In Kjelneset, concentrations above PNEC can be found up to ~52 hours (Figure 68). In Jakobsteinvika concentrations above PNEC can be found up to ~22 hours after release (Figure 69). Concentrations exceeding PNEC for more than 22 hours can be found in relatively large distances from the release sites for all modelled locations (size of area and time above PNEC vary between the locations). A 22 h + exposure to H_2O_2 concentrations above PNEC may induce effects in many species, depending on the concentrations. The illustrations show how many hours animals can be exposed to concentrations above PNEC, but it does not specify how much over PNEC the environmental concentrations are.

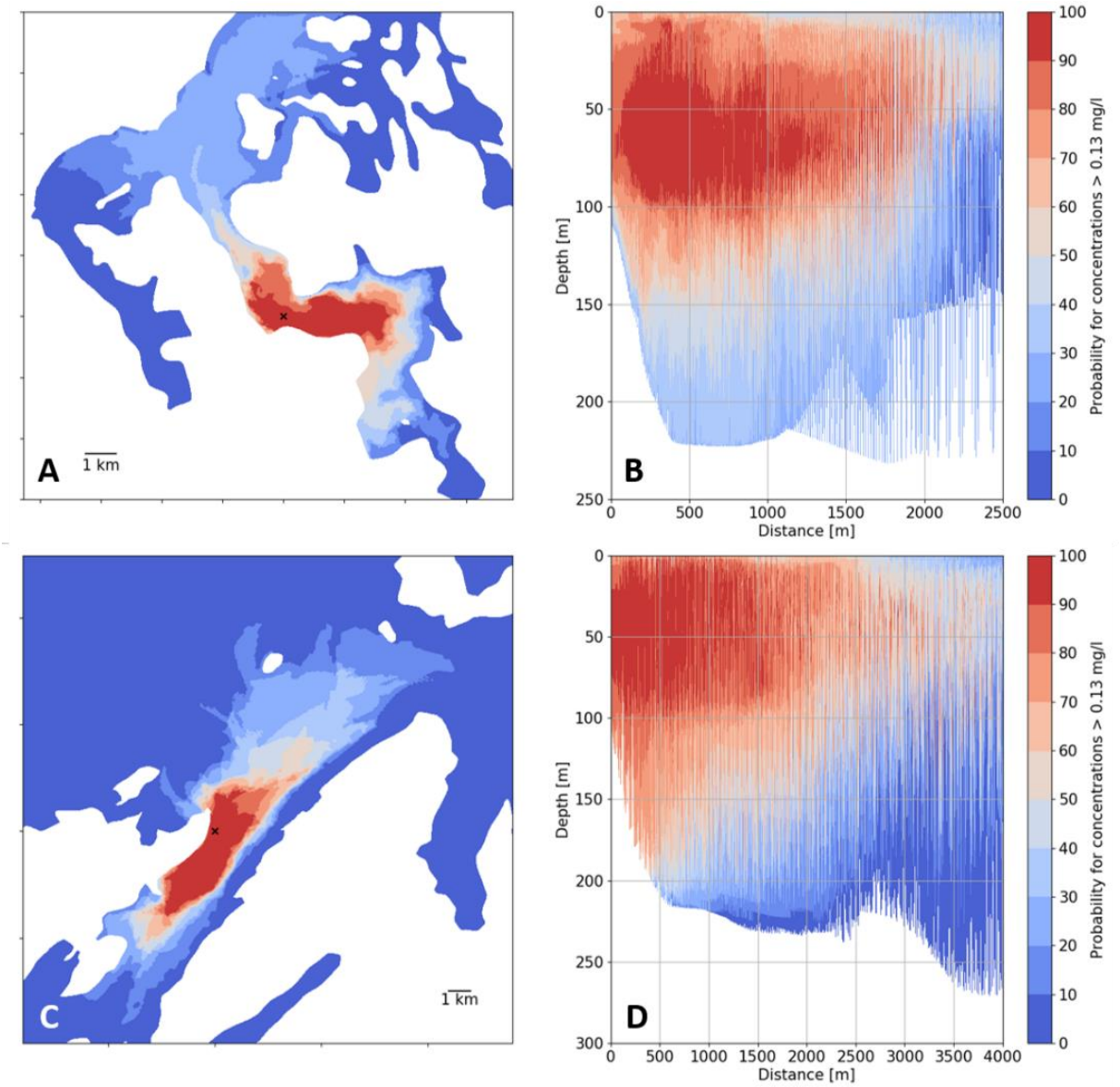

Figure 65. Probability of H_2O_2 concentrations (from 12 scenarios) above PNEC (0.14 mg/L) after delousing in 4 cages. A and B show probability for H_2O_2 concentrations above PNEC: relative to the surroundings (left) and the vertical distribution (right) for the location Austvika (marked by a black cross). C and D show the same for Jakobsteinsvika. NB! The probability will be lower than shown here when single cage releases are considered.

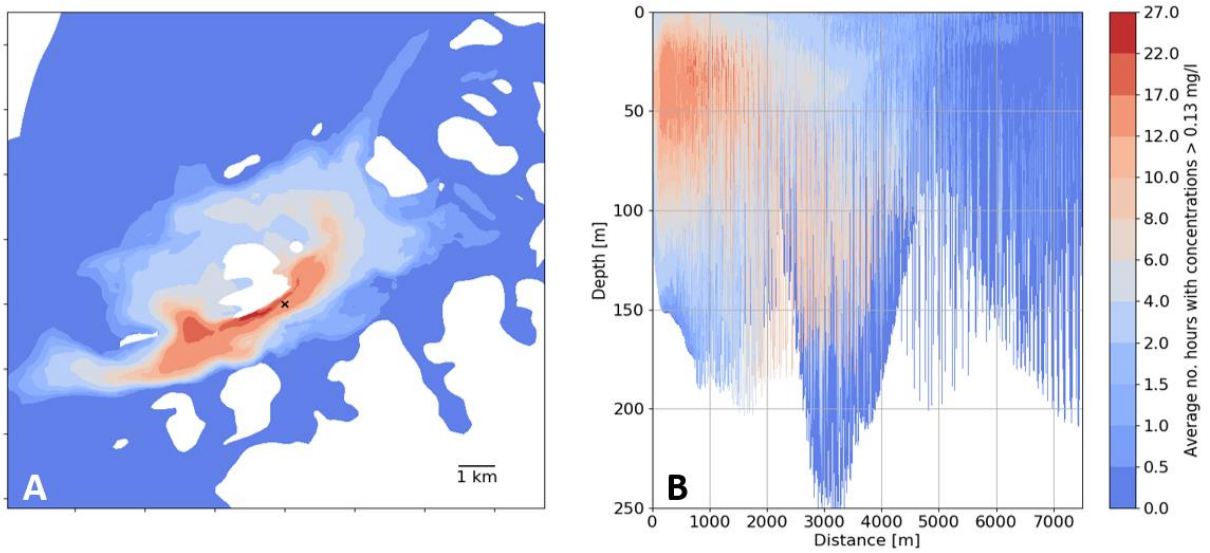

Figure 66. Average time with H_2O_2 concentrations (from 12 scenarios) above PNEC in Skjervøy after release from 4 cages (120 m). The horizontal distribution is plotted in A, the black cross shows the release site. The vertical distribution is plotted in B, by distance from the black cross.

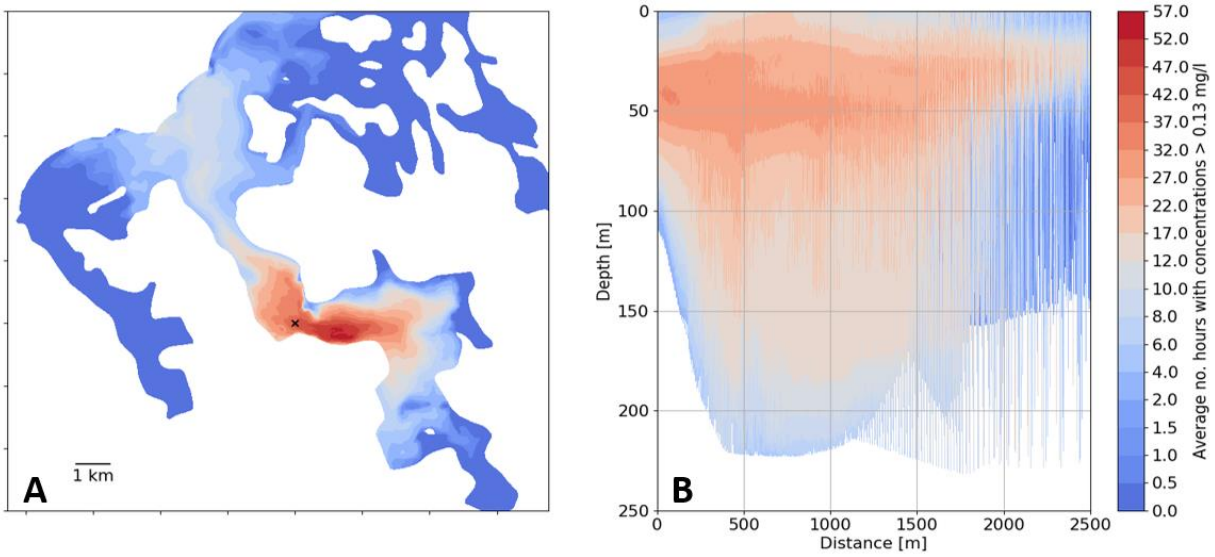

Figure 67 Average time with H_2O_2 concentrations (from 12 scenarios) above PNEC (i.e. between discharge concentration and PNEC) in Austvika after release from 4 cages (120 m). The horizontal distribution is plotted in A, the black cross shows the release site. The vertical distribution is plotted in B, by distance from the black cross.

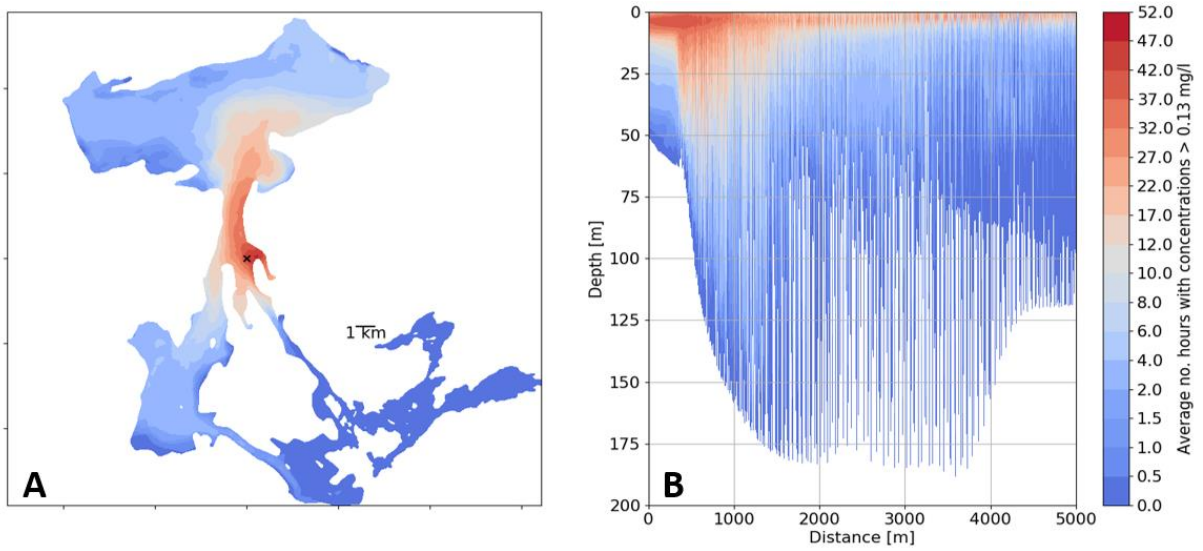

Figure 68. Average time with H_2O_2 concentrations (from 12 scenarios) above PNEC (i.e. between discharge concentration and PNEC) in Kjelneset after release from 4 cages (120 m). The horizontal distribution is plotted in A, the black cross shows the release site. The vertical distribution is plotted in B, by distance from the black cross.

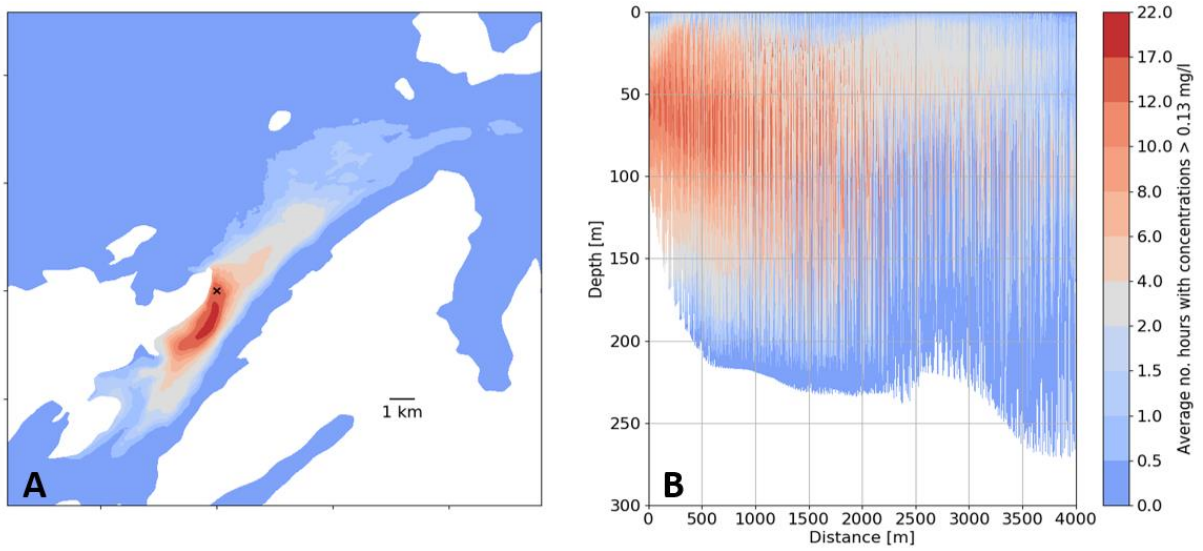

Figure 69. Average time with H_2O_2 concentrations (from 12 scenarios) above PNEC (i.e. between discharge concentration and PNEC) in Jakobsteinsvika after release from 4 cages (120 m). The horizontal distribution is plotted in A, the black cross shows the release site. The vertical distribution is plotted in B, by distance from the black cross.

Using a wellboat, the results show that the H_2O_2 concentrations above PNEC persist for a shorter time in the environment compared to direct releases from cages. Concentrations above PNEC can be found in up to ~22 hours after release from cages in Jakobsteinsvika, while after release from wellboat, concentrations above PNEC are present for ~3 hours. An exposure time of 3 hours can result in effects in some of the species listed in Table 6 - Table 8, depending on concentrations.

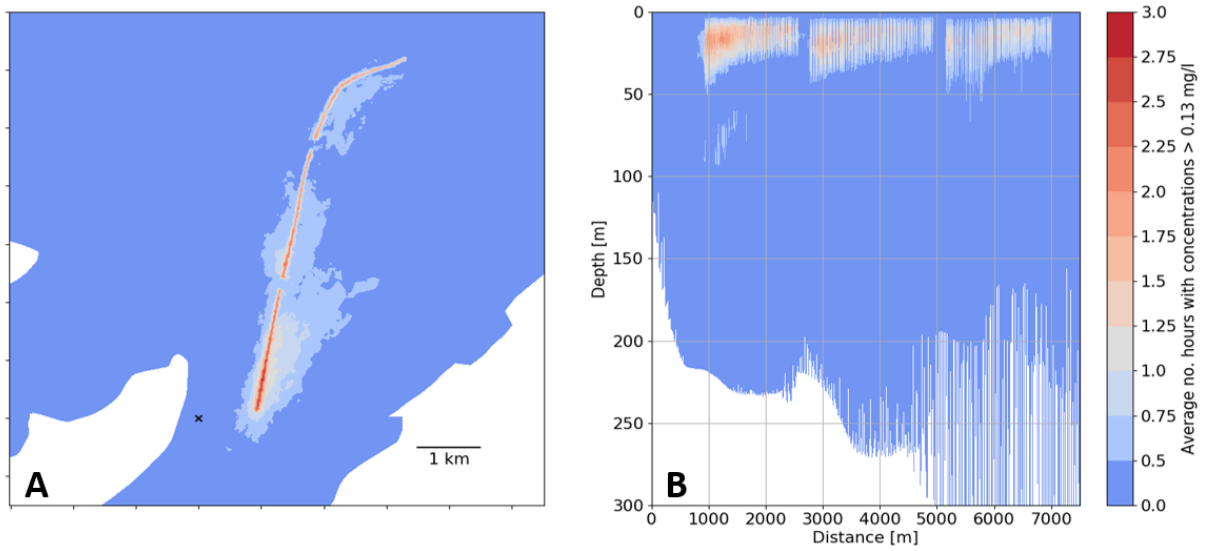

Figure 70. Average time with H_2O_2 concentrations above PNEC (i.e. between discharge concentration and PNEC) in Jakobsteinsvika after release from wellboat. The figure to the left show areas with concentrations above PNEC. The black cross shows the location of the fishfarm (release site in Figure 69). The figure to the right show vertical distribution by distance from the black cross.

In Figure Figure 71- Figure 74 average time with H_2O_2 concentrations above HC_5 is shown for the four modelling areas: Skjervøy, Austvika, Kjelneset and Jakobsteinsvika. The figures show that there is a high probability that concentrations above HC_5 can be present several kilometers away from the release site. There are however large variations between sites. In Kjelneset concentrations exceeding HC_5 can persist in the water column for up to ~14 h within an area reaching ca. 500 m from the release site (Figure 73), whereas the dilution is more rapid in the other locations, and especially in Jakobsteinsvika where concentrations above HC_5 can be found up to ~6 hours after release ca. 500 m from the release site (Figure 74). Concentrations exceeding HC_5 for more than 2 hours can be found in large distances from the release sites; ca. 3 000 m in Skjervøy, ca. 3 500 m in Austvika, ca. 2 000 m in Kjelneset and ca. 2 000 m in Jakobstensvika. A 2-hour exposure to concentrations equaling NEC may induce effects in the most sensitive species, especially if the exposure is repeated. However, for most species a longer exposure time is required for mortality to occur (see chapter 4.3.1.5). Exposure-time that will induce effects at HC_5-concentrations has not been calculated.

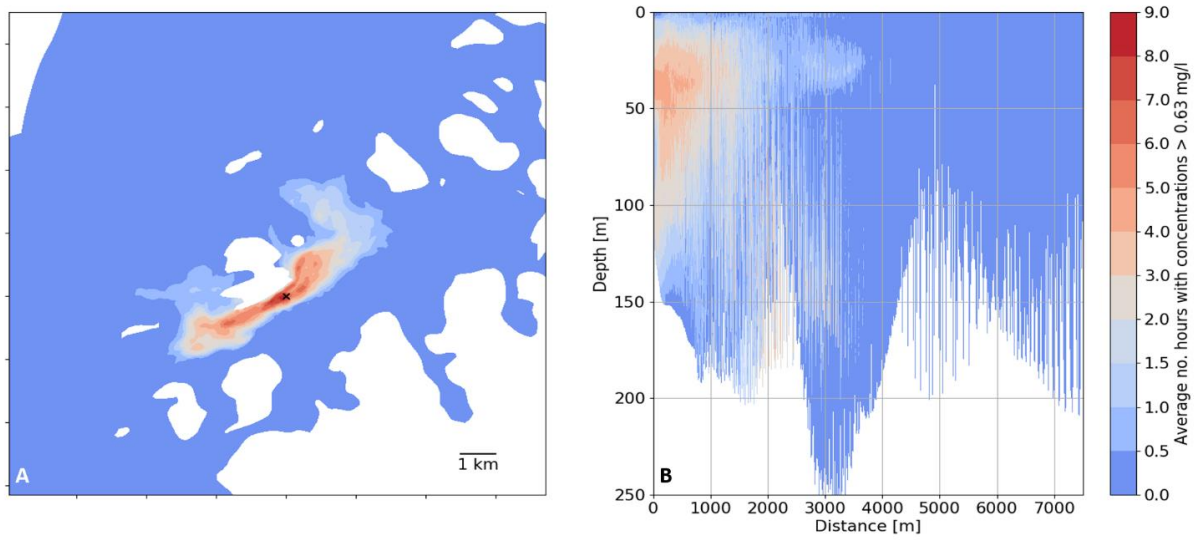

Figure 71. Average time with H_2O_2 concentrations (from 12 scenarios) above HC_5 (i.e. between discharge concentration and HC_5) in Skjervøy after release from 4 cages (120 m). The horizontal distribution is plotted in A, the black cross shows the release site. The vertical distribution is plotted in B, by distance from the black cross.

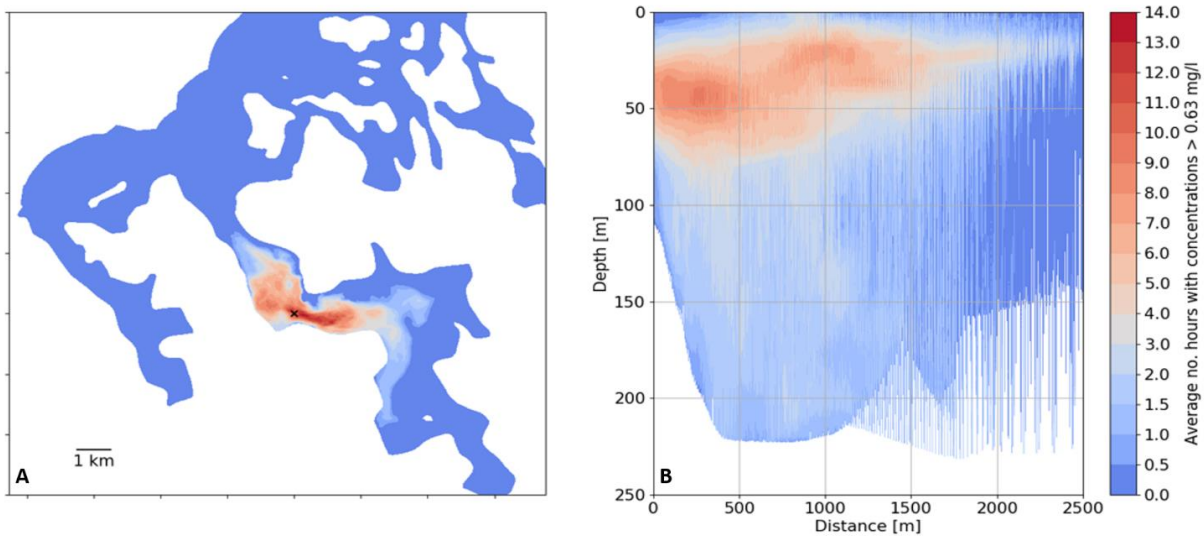

Figure 72. Average time with H_2O_2 concentrations (from 12 scenarios) above HC_5 (i.e. between discharge concentration and HC_5) in Austvika after release from 4 cages (120 m. The horizontal distribution is plotted in A, the black cross shows the release site. The vertical distribution is plotted in B, by distance from the black cross.

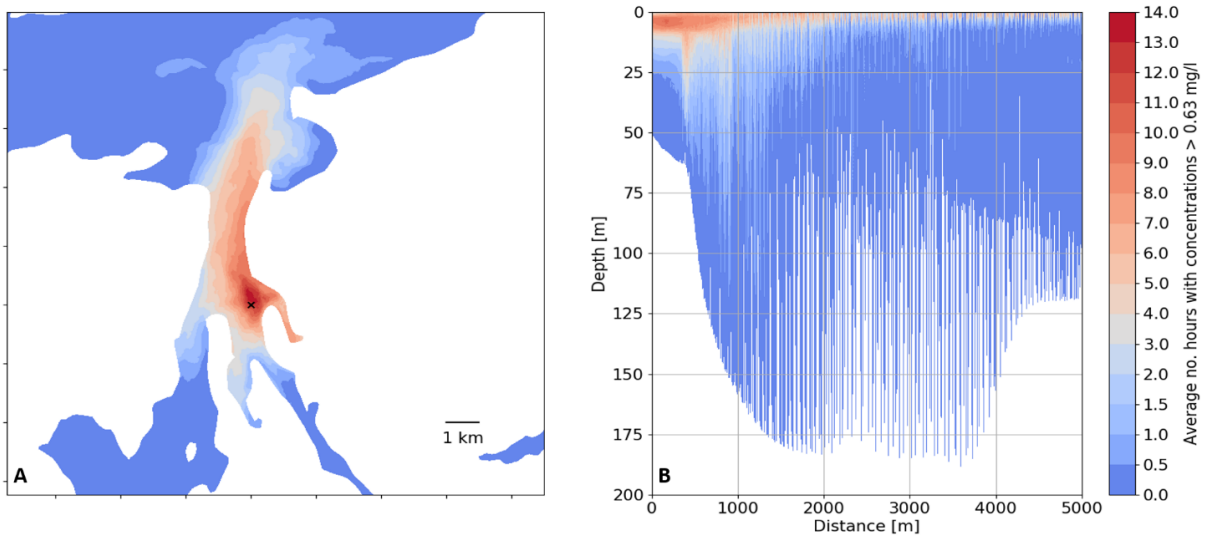

Figure 73. Average time with H_2O_2 concentrations (from 12 scenarios) above HC_5 (i.e. between discharge concentration and HC_5) at Kjelneset after release from 4 cages (120 m). The horizontal distribution is plotted in A, the black cross shows the release site. The vertical distribution is plotted in B, by distance from the black cross.

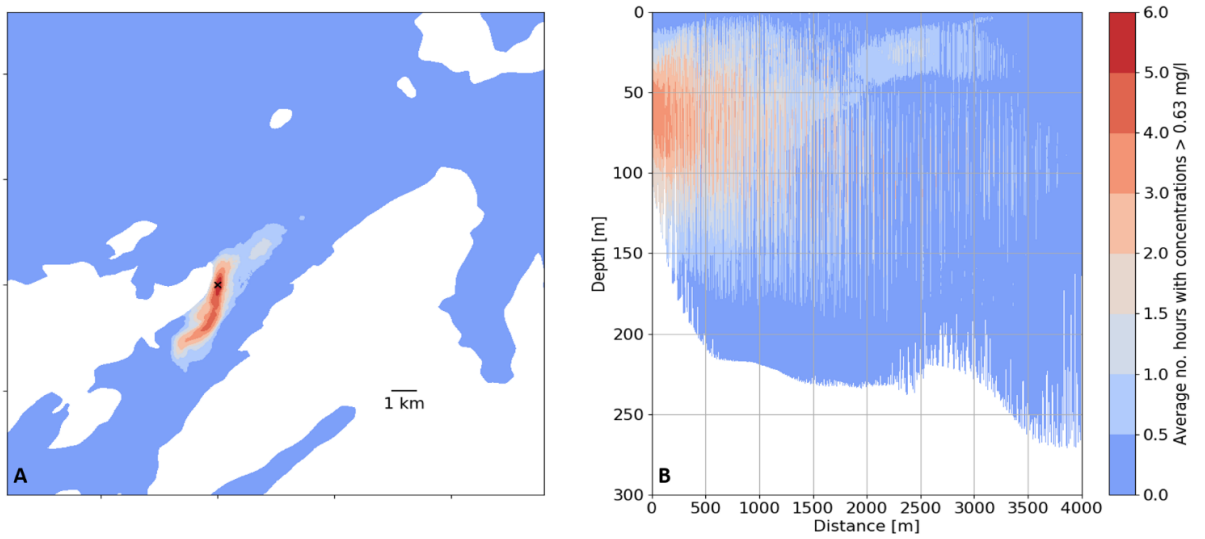

Figure 74. Average time with H_2O_2 concentrations (from 12 scenarios) above HC_5 (i.e. between discharge concentration and HC_5) in Jakobsteinsvika after release from 4 cages (120 m). The horizontal distribution is plotted in A, the black cross shows the release site. The vertical distribution is plotted in B, by distance from the black cross.

When wellboat is used the dilution happens very rapidly and the influence area and residence time is relatively small (Figure 75).

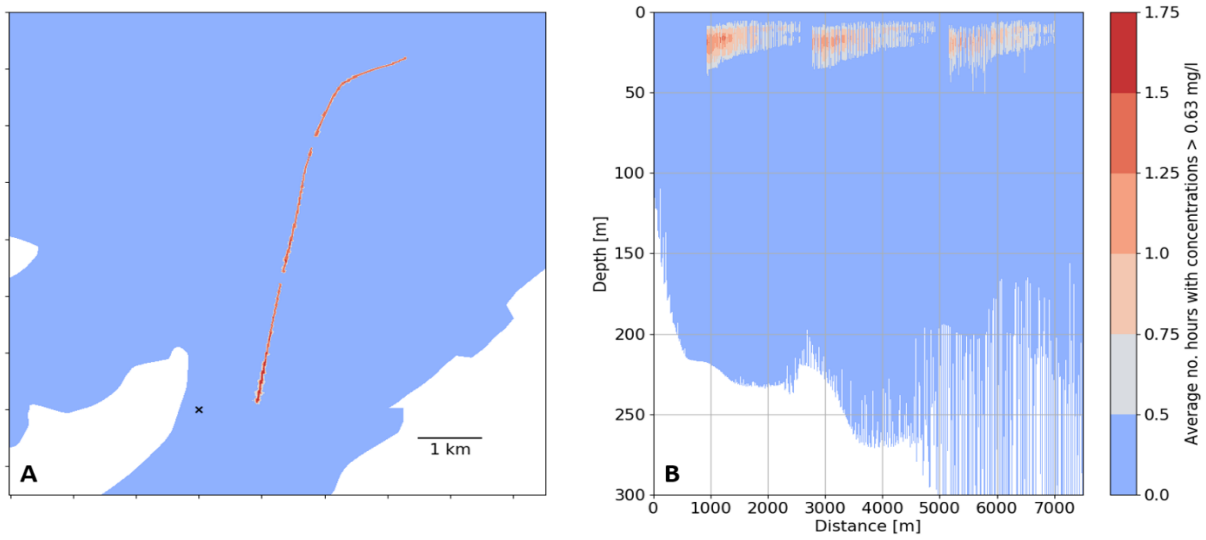

Figure 75. Average time with H_2O_2 concentrations above HC_5 (i.e. between discharge concentration and HC_5) in Jakobsteinsvika after release from wellboat. The horizontal distribution is plotted in A, the black cross shows the release site. The vertical distribution is plotted in B, by distance from the black cross.

7.3.1 Algae

Most data exist for freshwater algae, but some studies of marine algae have been performed. In general, marine algae seem to be sensitive to H_2O_2, with EC_{50} values ranging from 0.85 mg/L (*N. clostrium*) to 80.7 mg/L (*S. latissima*). All release of H_2O_2 is believed to come in contact with algae in the water column, and the area where negative effects may occur can for some species be approximately as shown in the figures illustrating the areas with concentrations above the PNEC derived from the SSD-curve. This means algae within large areas can be affected.

Sugarkelp has the highest recorded EC_{50} value for marine algae (Table 6). This is in agreement with results for invertebrates that shows that species living in the littoral zone, and experience large variations in environmental conditions, are more robust that species living in deeper areas. Kelp grows along the coast in shallow areas, i.e. down to ca. 30 m, and are likely to be affected if H_2O_2 is released in fjords. Kelp, algae and seaweed are important in the ecosystem, and if they are affected, cascading effects to other parts of the ecosystem may occur.

7.3.2 Invertebrates

Several species and phyla of marine invertebrates have been tested, i.e. crustaceans, mollusks, polychaetes, but most data exist for different crustaceans. As expected, crustaceans are most sensitive, but there is a huge span in EC_{50} values between different species, from 2.5 mg/L for *C. finmarchicus* to 2 520 mg/L for *Gammarus* sp. Also, different shrimp species have a higher EC_{50} value than the copepod *C. finmarchicus*. When it comes to effects of H_2O_2 the size of the organisms is believed to be important, as it probably affects the surface of the organisms (i.e. the carapace) (Hansen et al. 2017), and a bigger size of amphipods, and shrimp (volume:surface-ratio) compared to copepods could possibly explain differences in responses. Gammarus sp. are amphipods that lives in the tidal zone. This means that they are exposed to large variations in temperatures and salinity and therefore quite resistant to external factors. This may explain the low sensitivity to H_2O_2. However, another species living in the tidal zone,

Buccinum undatum (juvenile) has a NEC value between 10 and 100 mg/L so there are large variations between intertidal species.

C. finmarchicus, is a very important ecological species in Norwegian coastal areas, as the copepodite stages is an essential food source for juvenile fish (Runge & de Lafontaine 1996, Heath & Lough 2007). It has a LC_{50} value after 1-h exposure of 35 mg/L for adults (Escobar Lux 2016). The results from dispersal modelling show that concentrations above 35 mg/L can be present for more than one hour in areas up to ~1 km from the release site, so quite substantial effects can occur for *C. finmarchicus* and maybe also for other crustacean zooplankton species. Refseth et al. (2016) estimated a NEC-value of 10 mg/L for *C. finmarchicus*. Concentrations of 10 mg/L can be present up to ~ 5 km from the release site, depending on location. A concentration of 10 mg/L and an exposure time of 10 minutes resulted in total paralysis of copepods (VanGeest et al. 2014). Hence, if sub-lethal effects are considered into the risk assessment, the area associated to risk can be larger than indicated here.

Zooplankton has a wide distribution in the open water masses, and individuals lost in one area can rapidly be replaced by others moving in with currents. However, population effects can occur if a substantial amount are affected by H_2O_2. This may affect populations and may also lead to cascading effects to other ecosystem components that feed on zooplankton, e.g. cod larvae, herring, capelin.

In future studies, assessment of total volume of water where impacts occur (not volume from only one farm, but from the total number of farms in Norway) should be estimated to assess the lost biomass of *Calanus* and other crustaceans.

7.3.3 Fish

Fish are in general quite tolerant to short-term exposures to H_2O_2. This is of course necessary in order to use H_2O_2 in cages with fish. There are relatively few data on the sensitivity of different marine fish species, but *C. lumpus* has a LC_{50} value (24 h) of 167 mg/L, i.e. ca. 10 times diluted treatment dose. Eggs of Atlantic cod (*G. morhua*) are quite resistant, with an LC_{50} value of 342 mg/L. The chorion may have a protective effect preventing effects on embryos. However, this theory requires further research. Early larval stages may be more sensitive than eggs. The available data indicate that only fish that reside in very close vicinity to release sites may be affected if they don't swim away. The consequences for adult fish are probably limited, but larval stages with limited swimming capacity may be more sensitive and a release in/close to nursing areas may have negative impacts on local stocks. More data are required to test this hypotheses.

7.4 Risk for selected species

7.4.1 Deepwater shrimp

The deep-water shrimp is an important species in Norwegian fjord ecosystems, and a commercial resource. According to fishermen the shrimp stocks in Norwegian fjords has decreased substantially the past few years. The reason for this decline is unknown, but several factors may have contributed, such as climate changes, fisheries, and release of chemicals.

The shrimp lives just above the bottom, with periodic migrations into free water masses (grazing behavior) and yearly to shallower areas (spawning migrations). Thus, shrimp may be affected by H_2O_2 both in the pelagic zone and at the bottom (Figure 76). The results from our

studies shows that H_2O_2 will sink, especially during the winter when water masses are not stratified, and thus expose shrimp. The new model results show that concentrations above NEC for shrimp (23 mg/L) will be present for a short time at all the test locations. In Austvika and Kjelneset concentrations above NEC that last for ca. 2 h (time to reach mortality for shrimp) are estimated to occur at distances of ca. 100 and 50 m from the release site (Figure 77; Figure 78). In Jakobsteinsvika the dilution is more rapid and concentrations >23 mg/L is not expected to occur for more than a few minutes (Figure 79). However, these estimations are performed with a NEC value of 23 mg/L. As previously discussed, mortality of deep-water shrimp has been demonstrated at concentrations below the NEC of 23 mg/L established in Refseth et al. (2016). Mortality was observed after 2-h exposure at concentrations of 15 mg/L, and after 2-h exposure to 1.5 mg/L on 3 consecutive days, mortality was also observed (Bechman et al. 2019). Figure 76 - Figure 79 is based on NEC of 23. A lower NEC will give a higher risk. As previously discussed, different available toxicity data should be discussed when the data are used in risk assessment. The plots using data from Bechmann et al. (2019), yields a higher risk for effects on the shrimps, as shown in Figure 81.

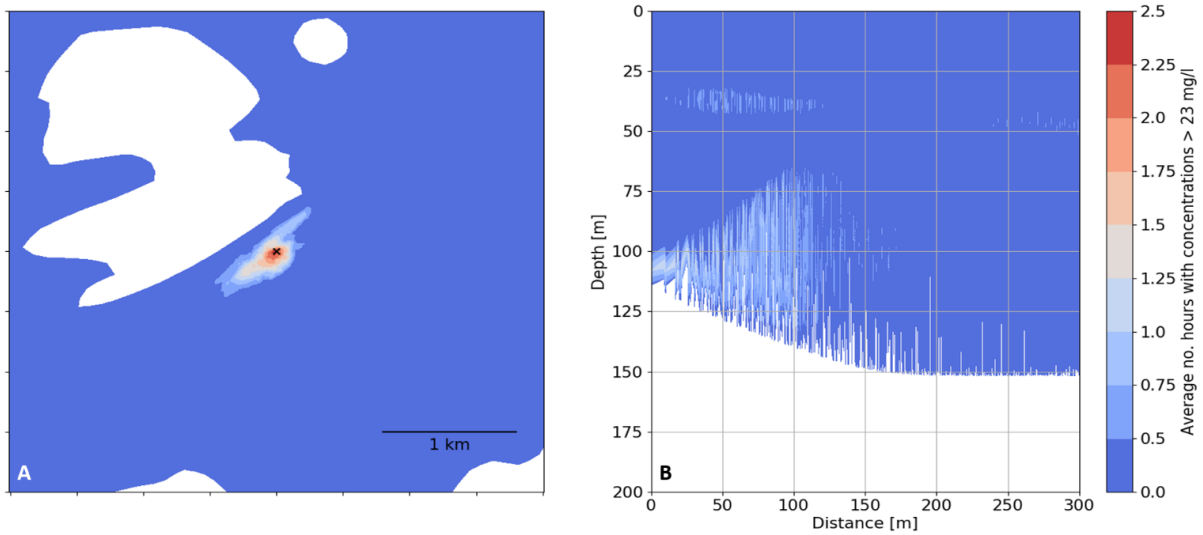

Figure 76. Average time with H_2O_2 concentrations (from 12 scenarios) above NEC for deep-water shrimp (P. borealis) in Skjervøy after release from 4 cages (120 m). The figure to the left show horizontal distribution, the black cross shows the release site. The horizontal distribution is plotted in A, the black cross shows the release site. The vertical distribution is plotted in B, by distance from the black cross.

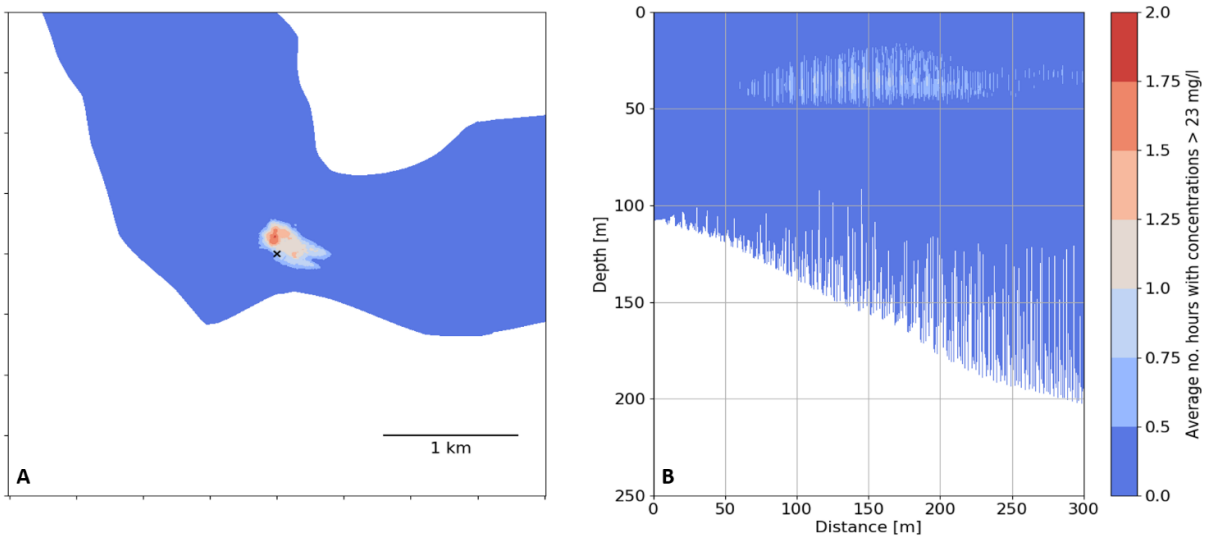

Figure 77. Average time with H_2O_2 concentrations (from 12 scenarios) above NEC for deep-water shrimp (P. borealis) in Austvika after release from 4 cages (120 m). The horizontal distribution is plotted in A, the black cross shows the release site. The vertical distribution is plotted in B, by distance from the black cross.

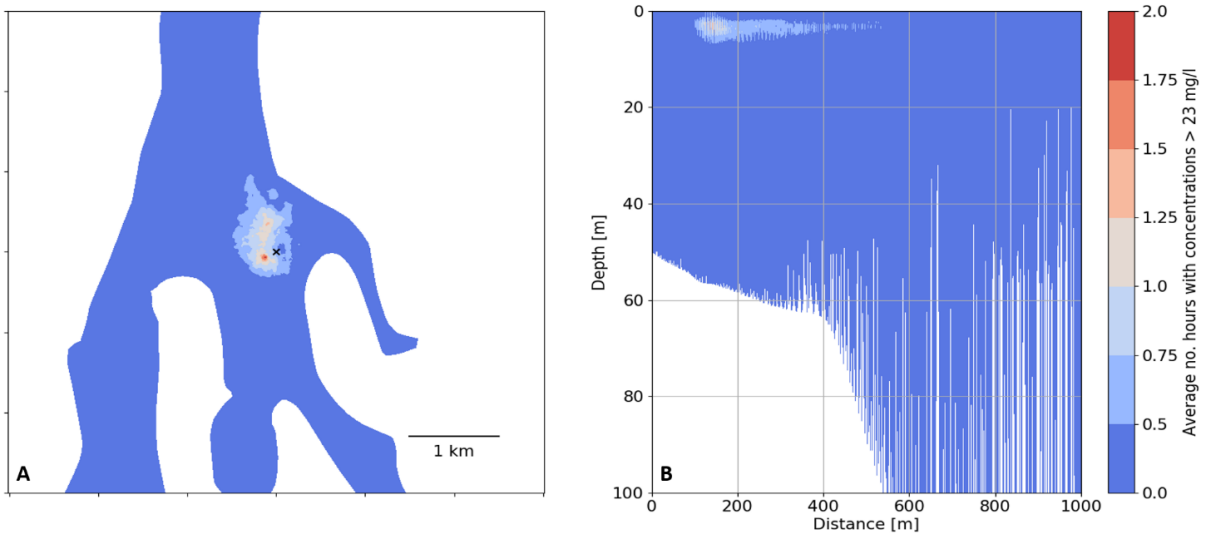

Figure 78. Average time with H_2O_2 concentrations (from 12 scenarios) above NEC for deep-water shrimp (P. borealis) in Kjelneset after release from 4 cages (120 m). The horizontal distribution is plotted in A, the black cross shows the release site. The vertical distribution is plotted in B, by distance from the black cross.

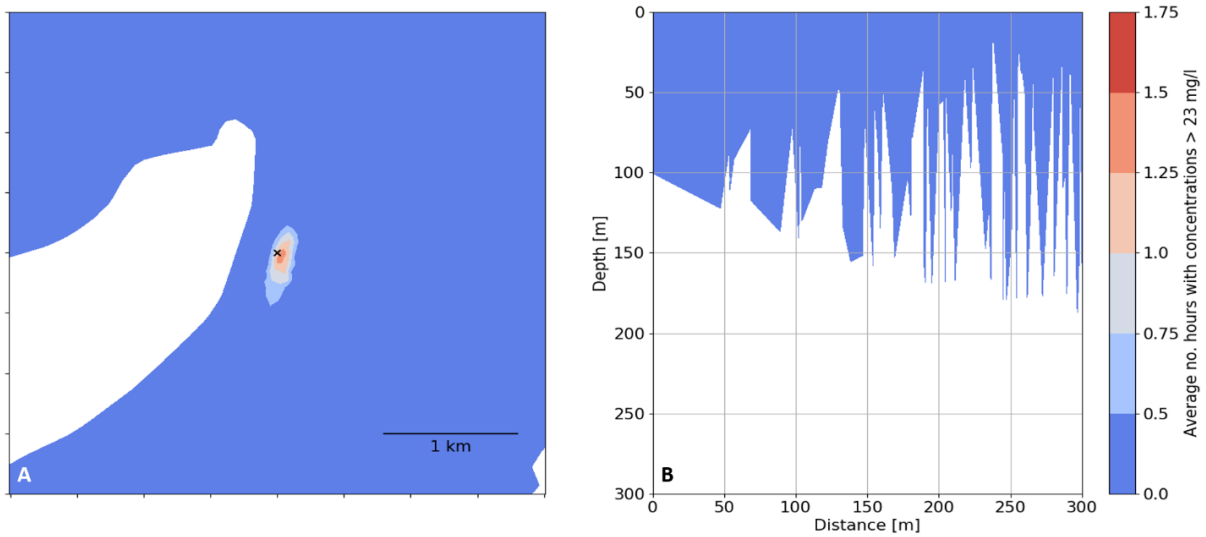

Figure 79. Average time with H_2O_2 concentrations (from 12 scenarios) above NEC for deep-water shrimp (P. borealis) in Jakobsteinsvika after release from 4 cages (120 m) with wellboat-volume (less than at the other locations). The horizontal distribution is plotted in A, the black cross shows the release site. The vertical distribution is plotted in B, by distance from the black cross.

When H_2O_2 is released from wellboat concentrations above 23 mg/L will be present for some minutes in the area in shown in Figure 80. The results indicate that the risk for adult shrimp is limited to areas within ~ 100 m from the treated cages. However, the illustrations do not show how much the concentrations will be over NEC. Short term exposure to high concertation may give other effects than short term exposure to low concentrations, obviously. Other ecotoxicological data, e.g. the data from Bechmann et al. (2019) will yield other results with higher risk than this illustration shows.

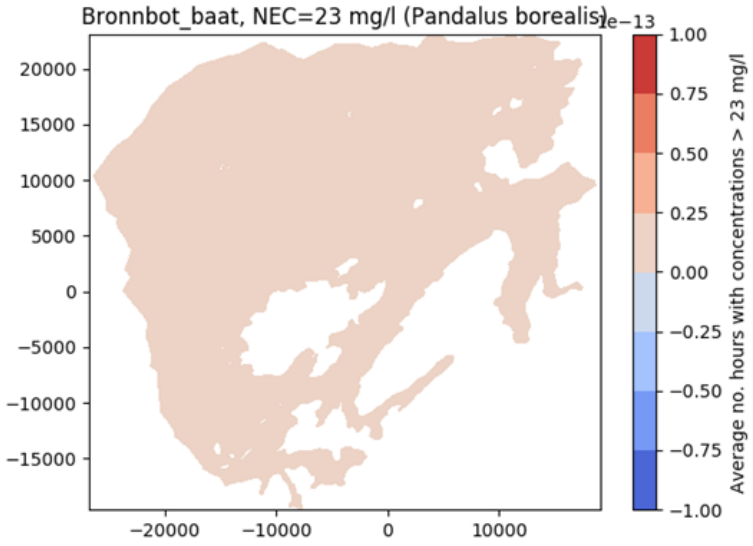

Figure 80. Average time with H_2O_2 concentrations above NEC for deep-water shrimp (P. borealis) after release from wellboat. Maximum concentration in deeper areas never exceeded 23 mg/L.

Figure 81 and Figure 82 show areas with concentrations comparable to those concentrations where mortality of shrimps was documented in Bechmann et al. (2019). Using these ecotoxicological data, results reveal that mortality of shrimps can occur several kilometres away from the release site. Shrimps can be exposed to different harmful concentrations at different depths and distances from the release site, as they perform migrations up and down in the water column.

Figure 81. Average time with H_2O_2 concentrations (from 12 scenarios) similar to those associated to mortality of deep-water shrimp (P. borealis) in Kjelneset after release from 4 cages (120 m). The horizontal distribution is plotted in A, the black cross shows the release site. The vertical distribution is plotted in B, by distance from the black cross.

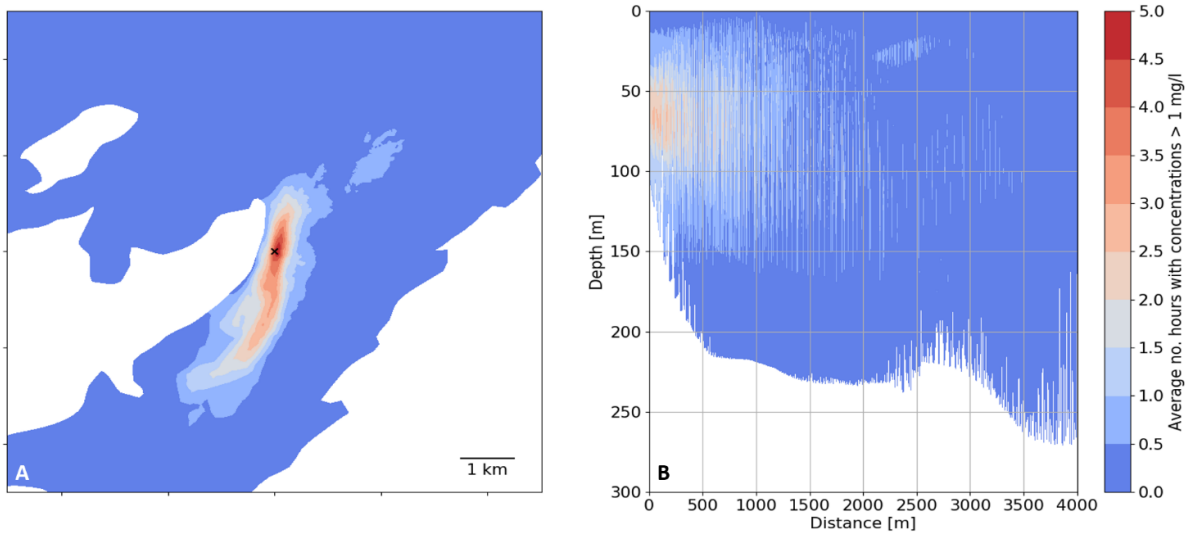

Figure 82. Average time with H_2O_2 concentrations above 1 mg/L in Jakobsteinsvika after release from wellboat. 1 mg/l is similar to concentration where mortality has been documented in deep water shrimps. The horizontal distribution is plotted in A, the black cross shows the release site. The vertical distribution is plotted in B, by distance from the black cross.

In summary, the result for deep water shrimps reveal that the risk for shrimps are low when using ecotoxicological data from Refseth et al. (2016), but that the risk is higher, and mortality can occur several kilometres away when using ecotoxicological data from Bechmann et al. (2019). As previously examined, it is well known that ecotoxicological data vary according to many different biological, physiological and physical factors, therefore it is not possible to assert that one result is more correct than the other, as both studies reflect the results according to the conditions the experiments were conducted.

Concern has been raised that delousing chemicals may cause shrimp to shred their eggs. In a recent project (Frantzen et al. 2019) effects of H_2O_2, azamethiphos and deltamethrin on egg-carrying shrimp were investigated. Both mortality and sub-lethal effects (behaviour, embryo development, and reproductive output) were studied for each chemical alone and in different sequential combinations. Generally, the results showed that the deep-water shrimp is sensitive to delousing chemicals, as some of the bath treatments killed the exposed individuals at highly diluted treatment concentrations. H_2O_2 concentrations in the range 1.6 – 16 mg/L caused no significant effects on egg carrying shrimp or on eggs/embryos. The most severe effect was

observed for deltamethrin, where 2 h exposure to 330 times diluted treatment dose (alone and in sequential use with H_2O_2 and azamethiphos) induced ~100% mortality within few days after exposure. Similar effects were not observed for H_2O_2 or azamethiphos. However, sequential treatment with H_2O_2 and azamethiphos (2 h exposure to each treatment chemical; 500 times diluted treatment dose) resulted in >50% mortality during the first week following treatment. The results for deltamethrin and azametiphos were comparable to those reported in Bechmann et al. (2019). Egg loss was not reported in either studies. For H_2O_2, Frantzen et al. (2019) did not observe mortality at concentrations as low as in Bechmann et al. (2019), as already discussed. No sub-lethal effects or loss of eggs in female shrimp could be related to exposure to any of the bath treatments. The results from the study indicated that the survival of the adult shrimp is most critical to produce viable offspring. The tested delousing chemicals appeared to have no effects on embryo development at the concentrations tested. However, if the egg-carrying shrimp dies the embryos will most likely die before hatching. Thus, the result indicates that bath treatments will exert their effects on eggs/embryos via their effects on the egg-carrying females rather than directly by damaging eggs/embryos. Newly hatched larvae may have a different sensitivity as they are small and don't have a protective chorion. Therefore, if size is a critical factor for H_2O_2 the NEC for larvae may be lower than for adults. With the data that exist today it seems to be better to use ecotoxicological values for adult shrimps rather than for eggs in risk assessment. However, different ecotoxicological results from the same species must be considered, as results may vary depending on factors previously discussed and shown for deep water shrimps. Sensitivity of shrimp larvae should be studied.

The current project provides valuable data about possible impacts of H_2O_2 releases from aquaculture facilities on shrimp. Nonetheless, there is a lack of knowledge when it comes to distribution of shrimp in fjord areas, natural fluctuations and impacts of other human activities, that makes it impossible to quantify impacts of H_2O_2 on stocks. As quantitative data on distribution becomes available, model results (PEC>PNEC) can be overlaid with distribution data to get an estimations of percent shrimp that may be affected. In our study, we only investigated possible impacts after release from 4 cages and releases from wellboats. The total marine area potentially affected along the Norwegian coast from different delousing chemicals over the years should be considered in order to assess how large areas have been/are potentially affected by delousing chemicals. When there are uncertainties in the data/lack of data needed to assess impact of populations, a precautionary approach should be taken.

7.4.2 **Atlantic cod**

For Atlantic cod data only exist for eggs. The calculated NEC-value is 147 mg/L, i.e. ca. 10 times diluted treatment concentration. Calculated exposure time for effects to occur at this concentration is 81.5 h (Table 11). As Figure 83 and Figure 84 show concentrations above 147 mg/L will only be present in less than 2 hours in Austvika and Kjelneset. In Skjervøy and Jakobsteinsvika the dilution is even more rapid than in Austvika and Kjelneset and exposure to concentrations above NEC will be less than 1 hour. Thus, it appears to be unlikely that H_2O_2 will stay in the environment long enough to have a negative impact on cod eggs. Newly hatched larvae and adults can be more sensitive than eggs. Institute of Marine Research has recently started a new project that will look at interactions between aquaculture activities and cod, so new data are expected in the coming years.

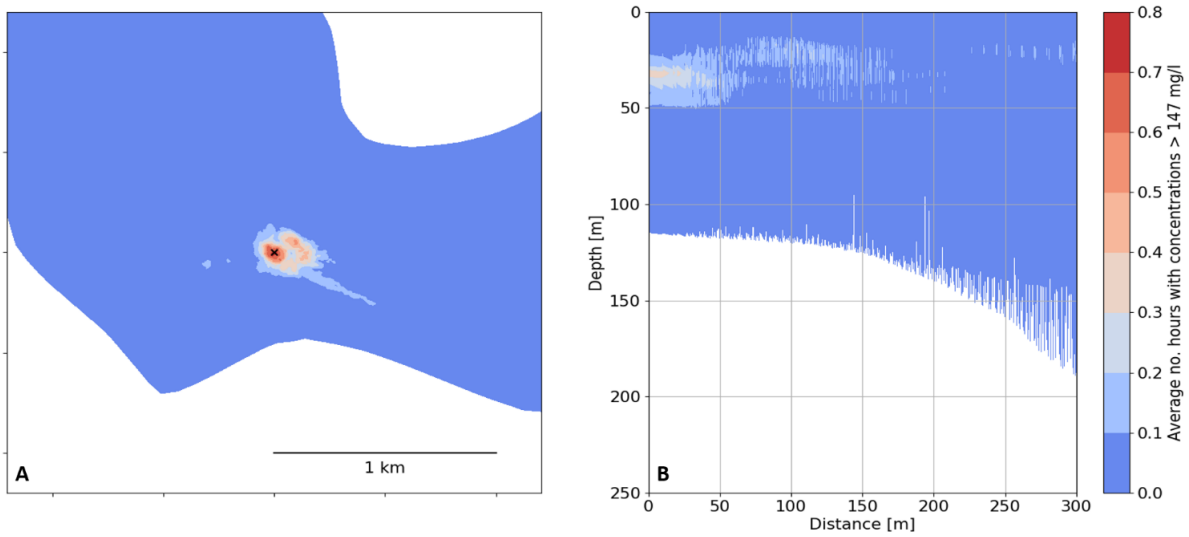

Figure 83. Average time with H_2O_2 concentrations (from 12 scenarios) above NEC for cod eggs (G. morhua) in Austvika after release from 4 cages (120 m). The horizontal distribution is plotted in A, the black cross shows the release site. The vertical distribution is plotted in B, by distance from the black cross.

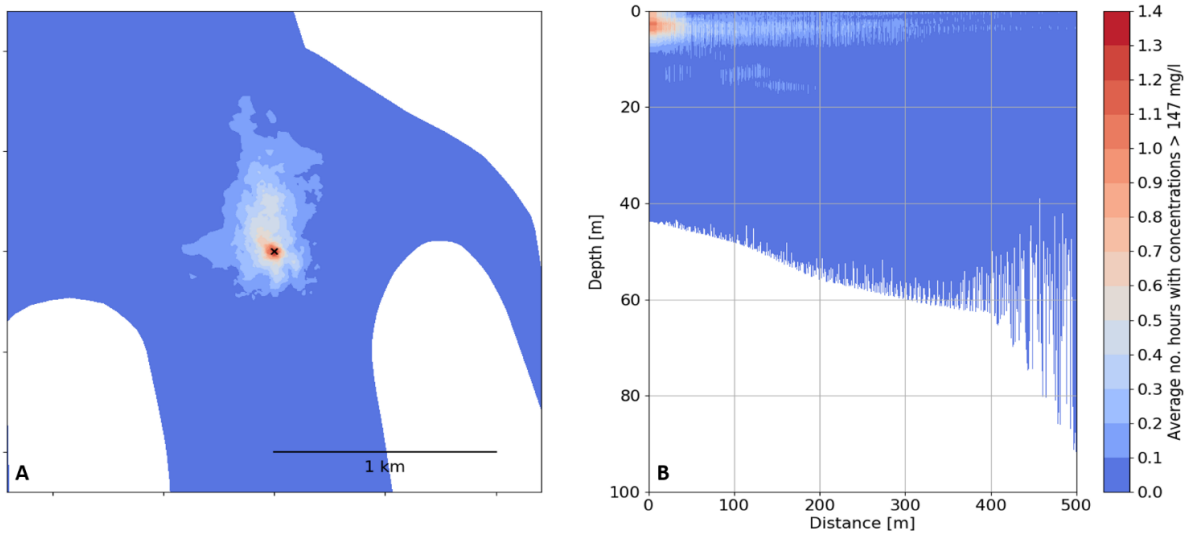

Figure 84. Average time with H_2O_2 concentrations (from 12 scenarios) above NEC for cod eggs (G. morhua) in Kjelneset after release from 4 cages (120 m). The horizontal distribution is plotted in A, the black cross shows the release site. The vertical distribution is plotted in B, by distance from the black cross.

7.4.3 Sugar kelp

In the study of Haugland et al. (2019), mortality and reduced photosynthetic performance for sugar kelp (*Saccharina latissima)* was demonstrated after a 1 h exposure to environmentally realistic H_2O_2 levels. Both LC_{50} and EC_{50} data are available in the study of Haugland et al. (2019). The study demonstrated delayed effects; it was essential to keep the plants in the laboratory for at least 7 days after exposure to be able to determine mortality with certainty. Juvenile *S. latissima* had an LC_{50} of 80.7 mg/L, which is less than 5% of the dose commonly

used at farms and emitted to the environment. A concentration of 85 mg/L caused an immediate 90% reduction in both PMAX and α. The EC_{50} was found to be 27.8 and 35.4 mg/L for PMAX and α, respectively. Average hours above concentrations similar to LC_{50} are plotted in Figure 85. The maps show that concentrations above those associated to both LC_{50} values are found in the environment, also at longer exposure time than the exposure time that caused the effects in the laboratory (1 hour), (both vertically and horizontally), suggesting risk of mortality and reduced photosynthetic performance in sugar kelp after discharge of H_2O_2.

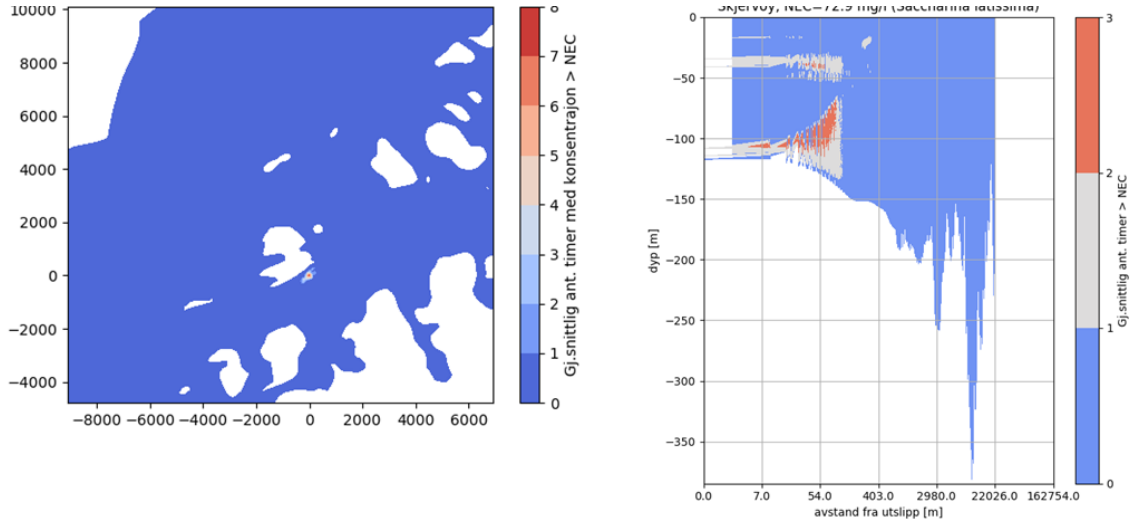

Figure 85. Average time with H_2O_2 concentrations (from 12 scenarios) above LC_{50} for S. latissima in Skjervøy *after release from 4 cages (120 m). The figure to the left show horizontal distribution and the figure to the right show vertical distribution.*

7.5 Conclusions

The ecotoxicological metrics defined in the current project and gathered from the literature reveal that the sensitivity to H_2O_2 exposure vary between species, but for all species except one, effects (mainly mortality and growth) occurred at concentrations well below the dose commonly used at fishfarms and emitted to the environment. When the ecotoxicological data are combined with data from oceanographic modelling results, the results reveal that there is risk for mortality in local ecosystems expressed via PEC>PNEC. The risk is reduced when a wellboat is used, but concentrations associated to mortality of a number of non-target species will still be present, but within a much smaller area and for a shorter time compared to discharges directly from the cage.

The results reveal that there is a risk for local ecosystem damage. It is still not known what impact this will have on a large scale when individuals/communities within limited areas is damaged, and we have not considered the total area which may potentially affected if we consider the total use of delousing agents in Norwegian fishfarms. There is a lack of methods to assess total risk for stocks or ecosystems on a larger scale.

Elucidation of the responses of populations to H_2O_2 requires integrative understanding of 1) the fate and transport of H_2O_2 (dispersal modelling), and 2) the biological effects linked to the toxicity of oil compounds (ecotoxicological tests), and 3) the transport, behavior, and

interactions of biota in the environment. The first two point have been investigated in this project, revealing risk to local ecosystems. However, transport, behavior, and interactions of biota in the environment are complex mechanisms that are not yet completely understood, but that are subject to research. In the future, ecological, ecotoxicological and dispersal models should be combined in order to estimate total, long-term risks related to releases of delousing chemicals.

However according to standardised risk procedure, risk reducing actions and further analysis should be taken when concentrations in the environment are exceeding threshold levels for effect (PNEC). When there are uncertainties in the data/lack of data needed to assess impact of populations, a precautionary approach should be taken.

8 Risk reducing measures

Environmental risk related to H_2O_2 can be reduced either by increasing dilution, by limiting emissions in time or space (if possible), or by a more rapid degradation. The breakdown products, H_2O and O_2 are harmless, so no accumulation of harmful compound will occur. Therefore, H_2O_2 is more environmentally friendly than compounds that may remain in the environment for a longer time or that may degrade to potentially harmful metabolites.

8.1 Dilution

The results from the dilution modelling have shown that there are some differences between releases from different cage sizes, but most important that there is a huge difference in environmental concentrations when a wellboat is used compared to delousing in cages. This is in agreement with results from field studies performed by Ernst et al. (2014), that documented significantly lower concentrations of azametiphos and deltametrin in environmental samples after delousing in a wellboat compared to in a cage. Flushing of wellboats has the effect of diluting the effluent, resulting in lower concentrations of pesticide reaching the environment. Our data confirm this. Concentrations and exposure times associated to effects in non-target species can still be found in the environment, but within limited volumes and with limited duration. Thus, treatments using wellboats will reduce the environmental impacts.

The size of cages will determine the volume that is release to the environment, and this is of course of importance for environmental concentrations. As shown in chapter 6.6 the maximum concentrations in the surrounding environment will be higher with a cage-size of 160 m compared to a cage size of 120 m. Thus, levels in the water can in some cases be lower that NEC-values with a 120 m cage and over with a 160 m cage. Also, the affected area and volume will be lower with a smaller cage. If the aquaculture facility is located close to sensitive resources, a reduction of cage size could be considered to reduce risk. Sinking happens after releases from both 120 and 160 m cages, but there are some differences in sinking depth (discussed in chapter 6), especially in the spring and in the autumn. In areas with potentially sensitive species near the bottom, e.g. in areas with shrimp or lobster, release time (season) and cage size can be critical. Releases in periods with stratified water masses will reduce the likelihood to expose benthic species to high concentrations.

Results from NORCE (Bechmann et al. 2019) shows that pulse-exposures may give more serious effects on e.g. shrimps than one pulse. When releases from a single cage is compared with releases from 4 cages (minimum 6 hours between each release) it appears that the effect of the previous release on the next is on average little, but for low concentrations it can still make a difference. Emissions from different cages may overlap and increase exposure concentrations. However, it is unknown if this in total will be more serious than if pulses are not overlapping.

8.2 Time and/or space restrictions

As mentioned in the previous chapter sinking depth varies between seasons and geographic locations, therefore local risk assessments should be performed for each location. If the goal is to limit horizontal distribution the winter is the most ideal season, while vertical distribution will be lowest in the summer season (some variations will of course occur between different regions). Knowledge about local species distribution will be essential to select the best period

to perform delousing for each location, if it is possible to choose time from a fish welfare perspective.

In spring high concentrations of algae will be present in the water column (spring bloom). Algae are in general sensitive to H_2O_2, and releases during the spring bloom may therefore affect large amounts of planktonic algae. This may have negative consequences for algae populations. On the other hand, it is possible that lost algae rapidly can be replaced by new growth in this season.

Many early life stages are present in spring and early life stages are generally known to be more sensitive to chemical compounds, compared to adults. Thus, delousing should be limited during the spring period.

In areas with large freshwater input water masses may be stratified year-round. It is important to be aware that this may give a larger horizontal, but a lower vertical dispersal. This may increase or decrease risk for the ecosystem, depending on the species that are present at the specific locations. Therefore, risk should be assessed for each location based on available knowledge about the local communities.

Wellboats can move to areas away from areas with aggregations of sensitive species prior to releases of chemicals. Using a wellboat is therefore the most important risk reducing measure defined in the current project.

8.3 Degradation

H_2O_2 degradation in natural aquatic environments is controlled mainly by photochemical, physical and biological processes. Given the broad range of process involved, the dynamics of H_2O_2 degradation in natural systems are still not entirely understood. Literature review presents varying reduction rates of H_2O_2, with half-life reported from almost an hour (Arvin & Pedersen 2015) to several days (Bruno & Raynard 1994) or even weeks (Lyons et al. 2014). Degradation has not been studied in the present project, but more knowledge about degradation under the physical and biological conditions that prevail in Norwegian fjord areas is necessary.

H_2O_2 is a very strong oxidant (electron acceptor), requiring an electron donor in the system to be reduced. In the marine environment, one of the most important electron donors is organic matter. Given that redox reactions are usually slow reactions (Stumm & Morgan 1996) without biological mediation, it is crucial to consider the bacterial role in the decomposition/reduction of H_2O_2 in the aquatic environment. The effects of dissolved organic matter on degradation should be investigated, as this can be a cheap and natural risk reducing method.

9 Overall conclusion

This project has provided a tool combining dispersal modelling with ecotoxicological data such as LC_{50}, EC_{50}, NEC and PNEC to determine the area of potential impact following de-lousing with H_2O_2. There was a great variation in the sensitivity towards H_2O_2 for the species tested, however, effects occurred at concentrations well below the concentrations normally used in the fish cage and discharged to the environment. The modelling shows that relatively high concentrations of H_2O_2 can occur close to the farm and potentially affect the ecosystem. Diluted concentrations, which can affect some species, will spread further away from the release site. The size of the influence area can vary due to variable currents, wind and stratification, as well with species diversity. We conclude that there is a risk for impacts on local ecosystems. However, the potential impacts on a larger scale is still not known. There is a need for combining ecological, ecotoxicological and dispersal modeling to evaluate risk at a larger scale.

The modelling shows that using a wellboat will reduce the environmental impact substantially, but there is still risk for harmful concentrations for some species.

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11 Appendix

11.1 Advection schemes

11.1.1 MPDATA:

Advection is the process of transporting a spatially inhomogeneous quantity and can be expressed by a differential equation: $advection = \vec{u} \cdot \nabla\phi$. ϕ is a scalar quantity (it could for example be temperature, salinity or H_2O_2), $\vec{u}$ is the velocity vector $\vec{u} = (u, v, w)$ where u and v are the horizontal- and w is the vertical velocity component. The gradient, $\nabla = (\frac{\partial}{\partial x}, \frac{\partial}{\partial y}, \frac{\partial}{\partial z})$, gives a measure of spatial differences of a quantity in a three-dimensional frame of reference. In one dimension, for instance in the vertical (z), one can write: $advection_{vert} = w\frac{\partial\phi}{\partial x}$. When finding the exact solution to this equation, we need to know the value of the variables everywhere in the domain. It is, however, impossible to have that knowledge, so we assume that it is sufficient to know their values somewhere in the domain, say every 20th meter. Let $z_i < z_{i+1}\ \forall i = 0,1,2,3,4,5,$ ... and ϕ_i be located at depth z_i. Define $\Delta z = z_i - z_{i-1}$ and rewrite the advection equation to get:

$$w\frac{\partial\phi}{\partial z} \approx w_i \frac{\Delta\phi}{\Delta z}|_i$$

Where the local derivative $\frac{\partial\phi}{\partial z}$ at point i is approximated from points nearby, $\frac{\Delta\phi}{\Delta z}|_i$. But which points should one use? A solution is to use the two neighboring points, ϕ_{i+1} and ϕ_{i-1} to get

$$advection = w_i \frac{\phi_{i+1} - \phi_{i-1}}{2\Delta z}$$

This is called the central scheme and is FVCOMs standard vertical advection scheme. It struggles in areas with very inhomogeneous scalar fields since this approximation of the gradient decouple odd- and even points. At even points ($i = 2, 4, 6$ ...) the gradient depends only on odd points ($i = 1,3,5$ ...) and vice versa. So-called "spurious oscillations" may occur and create unrealistic results. Such "unrealistic results" present themselves as areas where salinity, temperature and H_2O_2 are artificially produced by the model. FVCOM was therefore updated with an alternative scheme called "Multidimensional Positive Definite Transport and Advection Scheme" – MPDATA some years ago to avoid such unrealistic results. We had to alter this scheme to include precipitation and evaporation, as the scheme "out of the box" was restricted to applications where precipitation and evaporation has virtually no significance for the dynamics.

Freshwater can only be added to the model surface layer, which we do by noting that the salt is conserved

$$salt_{before} = salt_{after}$$

$$salt * volume_{before} = (salt * area * depth)_{before} = (salt * area * depth)_{after}$$

The grid area does not change with time, hence the area cancel out and we get

$$salt_{after} = salt_{before} * \frac{depth_{before}}{depth_{after}}$$

We allowed MPDATA to compute salinities as big (or small) as $salt_{after}$ in the surface layer.

11.1.2 A TVD scheme for horizontal advection:

Numerical schemes that do not produce spurious oscillations (lower order schemes) face problems with too much numerical diffusion, meaning that the model create more transport of the quantities across or along the current than the physics suggest it should. In other words, the quantities are artificially spread over a bigger volume than what happens in nature. Total Variation Diminishing (TVD) schemes are a mixture of lower- and higher order-schemes, which give results with less numerical diffusion than pure-lower order schemes give, and with none of the spurious oscillations related to the higher order schemes (Harten, 1983).

A scheme is TVD if the variation in the dataset does not increase with time, hence the total variation diminishes with time. Hartens define "total variation" at timestep n (where $n = 1,2,3,4, \dots$) as the sum of difference between points in space i ($i = 1,2,3,4,5, \dots$): $TV^n = \sum_{i=1}^{m} |\phi_{i+1} - \phi_i|$. A numerical scheme is TVD if $TV^{n+1} \leq TV^n$. To determine where to use the lower- or higher order scheme, Hartens introduced the smoothness parameter, r:

$$r = \frac{\nabla\phi_{i-\frac{1}{2}}}{\nabla\phi_{i+\frac{1}{2}}} = \frac{\phi_i - \phi_{i-1}}{\phi_{i+1} - \phi_i}$$

which control a weight function (ψ) that force the numerical model to use the lower order scheme in regions with sharp gradients (strong inhomogeneity), and the higher order scheme elsewhere. The transport from one grid point to another is determined by the flux through the control volume edge (wall) (see **Error! Reference source not found.**), which requires that t he advection scheme estimates the value of ϕ at the wall. Using the TVD scheme this is done by the equation:

$$\phi_{i+\frac{1}{2}} = \phi_i + \frac{\psi(r)}{2}(\phi_{i+1} - \phi_i)$$

In FVCOM, we use the superbee-weight function

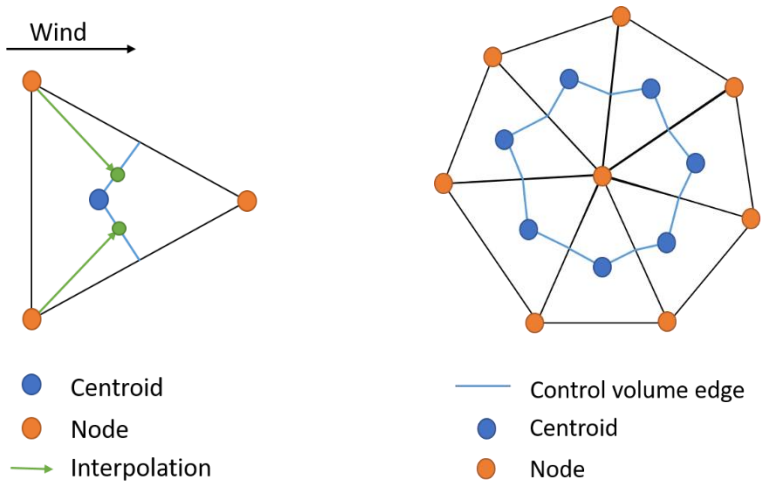

Figure 86 Left panel: FVCOM horizontal advection
Right panel: FVCOM grid

$$\psi(r) = \max([0, \min(1,2r), \min(2,r)])$$

11.1.2.1.1 Implementing TVD to FVCOM

FVCOM by default estimates ϕ at the control volume wall using the scheme sketched in the left panel of **Error! Reference source not found.**, and integrates it along the control volume e dge illustrated in the panel to the right.

The standard FVCOM procedure (Figure 86, left panel) is to identify the node upstream of the control volume wall (upwind) and use the gradient of ϕ at that node to interpolate ϕ to the wall. This is the "upwind scheme", and is a lower order scheme without spurious oscillations, but with more numerical diffusion than produced by a central scheme.

The TVD scheme for FVCOM (Figure 87) requires that we know two nodes upstream of the control volume wall. At first, we identify the node upstream of the calculation point. We thereafter find the distance $(\delta x, \delta y)$ between the upstream and downstream nodes and use it to define a "far-upstream node" as the point which lies $(-\delta x, -\delta y)$ upstream of the upstream node. In general, there will not be a node at this point. We therefore identify the node closest to the far-upstream node and use the gradient at that node to estimate the far-upstream value (ϕ_U), as illustrated in Figure 87.

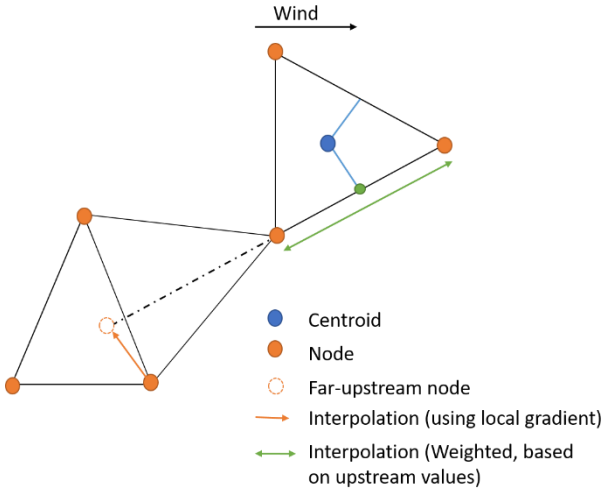

Figure 87 TVD scheme in FVCOM

We rewrite the equation for $\phi_{\frac{1}{2}}$ using

$$\phi_U = \phi_{i-1} \qquad \phi_C = \phi_i \qquad \phi_D = \phi_{i+1}$$

Where $\phi_U$is the far-upstream node, ϕ_c is the node directly upstream of the control volume wall, and ϕ_D is the node downstream of it. The smoothness parameter is then

$$r = \frac{\phi_c - \phi_U}{\phi_D - \phi_c}$$

So that the equation for ϕ at the wall becomes

$$\phi_{wall} = \phi_c + 0.5 * \psi(r) * (\phi_D - \phi_c)$$

11.1.3 Sinking of H_2O_2 released from a fish cage

To estimate the volume of the sinking H_2O_2, we need to know its shape. Experience suggests that it does not sink like a perfect sphere, but as a deformed one. One can define a deformation factor f and a characteristic thermal radius R to estimate its volume, $V = fR^3$. The rate of change of its volume is

$$\frac{dV}{dt} = Au$$

where A is the surface area of the sinking H_2O_2, and u is the entrainment speed - a measure of how fast surrounding fluid is entrained to the H_2O_2 mixture. The volume increases with time due to turbulent entrainment through the interface. Following (Morton, 1956), we parametrize the entrainment by assuming it is proportional to the vertical velocity of the sinking H_2O_2, thus $u = cw$. The surface area is $A = kR^2$, k being a proportionality constant.

We thereafter define $a = ck$ to arrive at an equation for the rate of change of volume following the sinking H_2O_2

$$\frac{dV}{dt} = aR^2w. \quad (1)$$

We can express equation (1) as a function of R and w by using $V = fR^3$ and the chain rule to get

$$\frac{dR}{dz} = \frac{a}{3f} \quad (2)$$

Following (Cushman-Roisin, 2018), we use $f = 2.54$ and $a = 1.9$. Thereafter Cushman-Roisin state that a sinking thermal moves 50% more mass than the fluid within the plume itself and assume that the water entrained to the thermal is stationary. Conservation of momentum is, under those circumstances, expressed as

$$\frac{d}{dz}(R^3w) = \frac{2R^3g'}{3w}. \quad (3)$$

When the water column is stratified, all fluid elements has a buoyancy when displaced vertically, hence the volume integrated buoyancy within the thermal changes as it entrains ambient water. Conservation of buoyance can be expressed as

$$\frac{d}{dz}(R^3g') = -N^2R^3, \quad (4)$$

where $N = -\frac{g}{\rho_0}\frac{d\rho}{dz}$ is a measure of the stratifications strength. $N = 0$ in homogeneous water and $N > 0$ in stably stratified water. We solve equations $2 - 4$ numerically using a finite difference scheme since there are no exact solutions to them if N is not a particularly well-behaved function. We release the thermal in FVCOM at the location where the thermal has reached the same density as the ambient fluid.