Simulation-based decision support system for delousing of Lepeophteirus salmonis

Erik Andreas Næstvold

December 2016

PROJECT THESIS

Department of Marine Technology
Norwegian University of Science and Technology

Supervisor: Professor Bjørn Egil Asbjørnslett

Preface

This thesis, and the problem described, arose through my work onboard MS Urter summer 2015. The vessel was working with active delousing of fish farms through the use of hydrogen peroxide. My job was to participate and help during the delousing operations as well as examine the procedures and mechanisms at hand, to find potential improvements. Several problems related to the delousing methodology were identified and were investigated over the span of my work period. The scope of these problems were quickly narrowed towards salmon louse developing resistance towards hydrogen peroxide, and the reason behind "sudden" mass death of salmon in relation to the delousing operations. These problems were not solved, and since then I have thought of the salmon louse problem and how it is developing and sought it as an interesting field with a lot of potential for development. Therefore my thesis and further master thesis will be towards this field. All illustrations in this thesis have been made by the author.

The thesis is written as part of the course TMR4560, where the whole course emphasizes on this thesis which is related to a field within my choice of specialisation: Marine resources and Aquaculture.

Erik Andreas Næstvold
Trondheim, December 16, 2016:

Abstract

Norway's aquaculture industry has through the last decade experienced an explosive growth. Total produced salmon biomass for 2015 had doubled compared to 2007 and the net export value tripled. The industry seeks to grow even further, and by 2050 it is sought to produce five times the biomass compared to 2015. This goal is highly dependant on what measures have been developed to reduce the environmental impacts of aquaculture, as no new regular production licenses are stated from the government until the problem has been sufficiently dealt with. This environmental problem is mainly related to the salmon louse, lepeptheirus salmonis, and its impact on wild and stocked salmonids.

Lepeptheirus salmonis lives as an ectoparasite on salmonids, where it feeds on mucus, tissue and occasionally blood. The impacts on the salmonids is tissue damage which increases its susceptibility for secondary infections, reduces overall health, and induce stress. Which in addition to other health aspects may lead to death. The vast numbers of salmon along the Norwegian coast has made the spread of salmon louse readily due to the large number of potential hosts. There has been developed several methods to combat the salmon louse, and the salmon louse has been estimated to cost the industry 2.5 billion [NOK] in direct delousing costs and 7 billion [NOK] through direct and indirect costs, yearly. The salmon louse is still a large problem for the industry and has been the main focus in this thesis.

In order to develop solutions to the salmon louse problem, it is important to understand the very nature of the problem. Most of the work put down in this thesis has been devoted to the section describing the salmon louse and the methods that have been developed against it.The lepeptheirus salmonis molts through eight stages, where its reproduction and host attachment stage is of extra interest. The many methods developed to combat the salmon louse have been categorized through its usage, constraints and applicability.

Through the gained knowledge of the lepeptheirus salmonis and available delousing measures, a simulation model has been developed. The simulation model has as its purpose to estimate salmon louse abundances arising from copepodite pressures generated at external sites, and estimate the effects from using the different delousing methods at hand. The model is constructed in a generic manner, readily enabling implementation of fish farm sites and vessels with delousing capabilities. The simulation model seeks to generate information which will help salmon production companies in their decision making in regards to coping with the salmon louse. Through estimating the effect and consequences of their planned measures on a short and long term basis. It also works as a delousing methodology manual which presents the applicability and constraints of the different delousing measures available in an objective fashion, suited for their specific location.

The validity of the simulation results is of now poor, there are too much uncertainty linked to the variables which form the basis of the results. The model however is functional, and by implementation of realistic variables would form good estimates. Developing these variables would take a considerable amount of time and research. Suitable task for a master's thesis.

Sammendrag

Norsk havbruksnæring har gjennom det siste tiåret opplevd en eksplosiv vekst.Totalt produsert biomasse av laks tilsvarte i 2015 dobbelt så mye som i 2007, og netto eksportverdi det tredobblede. Næringen ønsker ytterligere vekst, en vekst på fem ganger produsert biomasse i 2050 i forhold til 2015. Dette målet er svært avhengig av hvilke tiltak som blir utviklet for å redusere miljøbelastningene havbruk forårsaker i dag. Da ingen nye ordinære konsesjoner blir utstedt før miljøbelastningene relatert til oppdrett er tilstrekkelig håndtert. Dette miljøproblemet er hovedsaklig knyttet til lakselusen, lepeptheirus salmonis, og dens innvirkning på laksefisken, både vill og i merd.

Lepeptheirus salmonis er en ektoparasitt som lever på laksefisk, hvor den ernærer seg på slim, vev og tidvis blod. Innvirkningen den har på laksefisk er vevsskade som øker resulterer i økt motagelighet for sekundære infeksjoner, det reduserer generel helse og forårsaker stress som kan føre til død. Det store antallet laks langs norskekysten har gjort spredning av lakselus kritisk grunnet det store antallet potensielle verter. Det er utviklet flere metoder for å bekjempe lakselus, og lakselusa har blitt anslått til å koste bransjen 2,5 milliarder [kroner] i direkte avlusnings kostnader og 7 milliarder [NOK] gjennom direkte og indirekte kostnader i 2015. Lakselus er fortsatt et stort problem for bransjen og har vært hovedfokuset i denne oppgaven. For å kunne utvikle løsninger for å begrense lakselusas negative påvirkning, må en ha forståelse for hvordan lakselusen oppfører seg og dens biologi. Brorparten av arbeidet lagt ned i denne oppgaven har vært relatert til beskrivelsen av lakselusens mekanismer og biologi, samt redegjørelsen for de eksisterende tiltakene mot lakselusen som finnes. Lakselusens livssyklus består av åtte faser, skilt ved et skallskifte fra en fase til den neste. Det har blitt lagt ekstra vekt på den adulte fasen der den har mulighet til å reprodusere seg, samt fasen der den er betegnet som smittsom. Når det kommer til de forskjellige tiltakene mot lakselusen har disse blitt kategorisert etter virkemåte, bruksperiode samt restriksjoner i anvendbarhet.

Gjennom kunnskapen som har blitt opparbeidet om lakselusen og tiltakene mot den, har det blitt utviklet en simuleringsmodell. Denne modellen har som formål å estimere lusekonsentrasjoner som oppstår i merder som et resultat fra eksternt genererte lusepress, samt estimere effekten ved bruk av de forskjellige tiltakene som er tilgjengelig. Modellen er laget generisk, slik at det er lett å legge til oppdrettsanlegg og avlusingsfartøy inn i modellen. Simuleringsmodellen har som intensjon å gi oppdretteren beslutningsstøtte gjennom simuleringsresultatene. Dette ved at modellen estimerer effekten of konsekvensene av planlagte tiltak på lang og kort sikt. Modellen kan også brukes som en manual for valg av riktig avlusingsmetode for den spesifikke lokalitet. Ved at den presenter hvilke tiltak som er tilgjengelige og når det er fordelaktig å bruke det ene tiltak framfor de andre.

Per nå er variablene brukt i modellen preget av for mye usikkerhet til at estimatene kan bli brukt til beslutningsstøtte. Modellen er imidlertid funksjonell, og ved å implementere mer realistisk variabler danne gode estimater. Utviklingen av disse variablene vil kreve en betydelig mengde tid og arbeid. En arbeidsmengde godt egnet for en masteroppgave.

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1 Introduction

Seafood is Norway's third biggest export after oil and gas. It stood for an export value of 74.5 billion [NOK] in 2015 where aquaculture was amounted to 67% of the seafood export value (Fiskeri og Kystdepartementet). On world basis Norway is the biggest producer of Atlantic salmon. The Norwegian government stated in 01.01.2015 that it intended to expand the aquaculture industry even more, and by 2050 increase the sales value from aquaculture by a factor of six. This will demand a production of five million tonnes of Atlantic salmon and rainbow trout, five times higher than today's equivalent production(Aspaker, 2015). The government simultaneously states that that growth is dependent on several aspects, where the most governing is the environmental aspect of aquaculture. Further growth will not be possible before the environmental challenges are handled, which we do not know how to per date. Physical interpretation of this constraint is the fact that no new regular licenses will be stated before the environmental aspects on a nationwide level has been dealt with satisfactory. The governing problem of the environmental aspect is the salmon louse.

Salmon louse, lepeptheirus salmonis, is stated to be the largest hinder in further expansion of salmon production in Norway. It is estimated to cost the industry 2.500 million [NOK] annually (2015) (Iversen et al., 2015) in direct delousing costs. And estimated to cost 7 billion [NOK] in direct and indirect costs such as loss of potential biomass. The salmon louse is a crustacean which lives as an ectoparasite on salmon in salt water. Attached to the salmon, the salmon louse inflicts skin damage with subsequent osmoregulatory problems and is thus prone to secondary infections. Large quantities of salmon lice at single locations also oppose a serious threat to the wild stock, as it generates a louse pressure which especially smolt is susceptible for. Several measures have been initiated in order to control and remove the salmon louse. One can categorize the methods as preventive and active. Preventive methods are persistent methods like closed cages, on-shore facilities and use of cleaner fish. Active methods which will be the main focus of this thesis and are distinguished between in-feed treatment, bath treatment and mechanical delousing methods.

1.1 Background

Today Salmon louse is the biggest constraint in Norwegian Atlantic salmon production. The Salmon louse is being resistant to more and more of the delousing methods, as the cheapest method has had a habit of being over-used over a longer period of time until it does not have sufficient efficacy. First then the salmon producer heads towards the more expensive method in order to delouse. This in turn may lead to several locations with louse being resistant to several delousing methods.

Salmon louse has co-existed for a long time together with the wild stocks of Atlantic salmon. However, with the large densities of salmon present at salmon production facilities the possibilities for massively increased salmon louse exposure has increased dramatically. This again is proven to affect the wild stocks in a negative manner. There are almost no coastal waters along the Norwegian coast that does not have some forecasted copepodites present at any given time. Mainly due to the many "infected" salmon facilities which then expose surrounding waters with salmon louse through the ocean currents.

The salmon louse problem has had a habit of being viewed as a local problem. Where each farm site has the responsibility to maintain a low level of salmon louse. This focus neglects the very nature of the salmon louse and how it spreads. Research has concluded that salmon louse rises from copepodite pressures developed at external locations to the farm site. Which emphasizes the importance of treating the louse problem more as a regional problem, not only as a local one.

1.2 Production process of Atlantic Salmon

It is important to understand the production process of atlantic salmon, as the biggest biomass production is done in the mariculture stage of the production process. It is in this stage it is prone to external effects, such as the salmon louse. This stage is still mostly done in open net pens, regardless of external negative inputs, mainly due to lack of any better alternatives. Better alternatives cost vice that is, production of a kg biomass in mariculture environments is much less costly than one produced in a closed confinement. However, as the operational costs rise together with the price per kg, closed cages/onshore production has increasing opportunity to become a commercially viable option.

Production of farmed Atlantic salmon consists of four governing parts. Breeding, hatchery, production and live fish transport and slaughter/processing. The whole process from hatching until eatable salmon takes approximately two and a half to three years, given that the slaughtered salmon weighs around 5 kg. Breeding is done on basis of selected individuals from the wild stock, where characteristics as growth rate, maturation, tenacity, shape, size and fat levels are the governing characteristics for further breeding. Characteristic breeding has been done since the 70's in Norway, and is under constant development. The Atlantic salmon is anadromous, spending its first part of the lifecycle in fresh water and adult life in salt water. Therefore, the hatchery part until the salmon has begun its smoltification process is done onshore in fresh water. The eggs lay approximately 60 days at water temperatures of 8 Celsius before it hatches. After 10-16 months in fresh water the salmon has most likely become smolt, and is ready for the production phase in mariculture. It is important to screen the salmon when it is starting to become smolt, as if it does not experience a salt water envirironment after becoming smolt, the process can be reversed. Demanding it to stay further in fresh water until it becomes smolt again, delaying the hatchery phase which is costlier per generated kg of mass compared to the mariculture phase. The smolt can be separated into two groups dependant of when it is ready for mariculture. Stocking of 0-yearolds is normally done in September and October, this is after approximately nine months in the hatchery. 1-yearolds stocks around may after approximately 15 months. This is due to the biological factors causing the mariculture only being able to receive new stocks of salmon over a limited time two times a year.

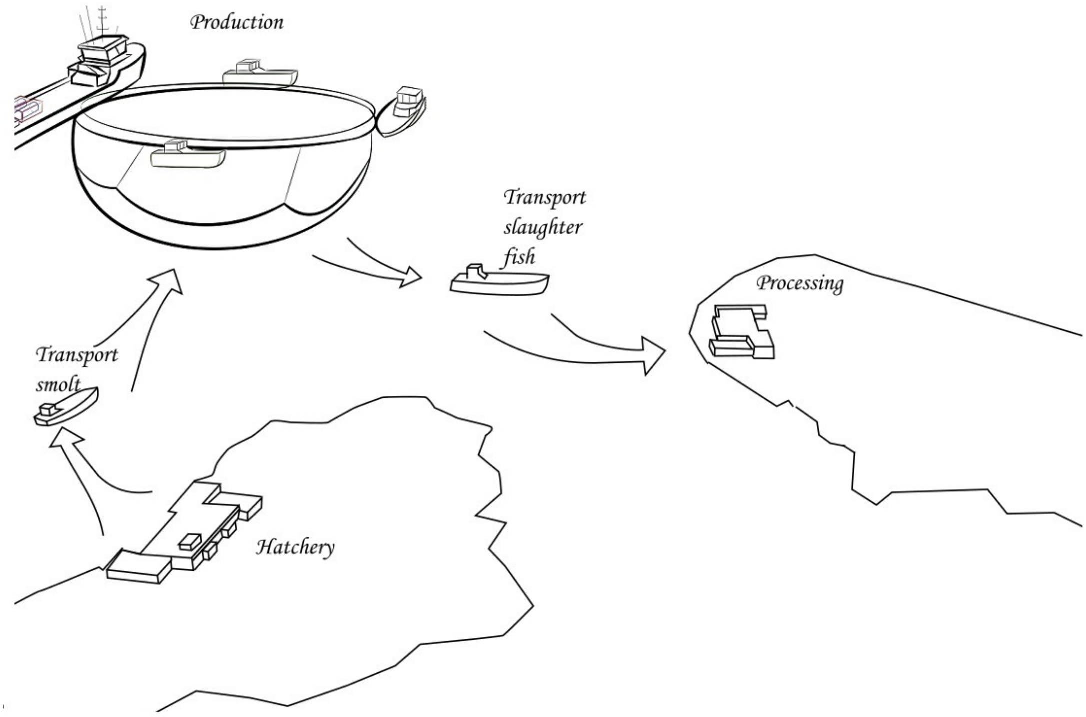


Figure 1: Production process of atlantic salmon

In Norway, the production of salmon is normally done in mariculture, mostly at fish sites consisting of fish cages. The smolt is stocked in when it has reached a weight of between 50-250 gram, however due to recent limitations larger and larger smolt is stocked to reduce the mariculture duration. The number of fish per stock is normally between 30.000 and 200 000 depending on the size of the fish cage. 200 000 is also the maximum allowed number of salmon to be stocked in one enclosure by Norwegian law, posing a problem for newer fish cage models. During the mariculture phase the salmon is fed pellets consisting of a mixture mainly of animal and vegetable products. The governing ingredients are fishmeal and fish oil. The salmon does not produce omega-3 by itself, rather stores it from the food it has consumed. Therefore, with poor feed the finished product may contain no omega-3. It is fed until it reaches desired weight varying from three to nine kg depending on the demand. 4-5 kg fish is usually achieved after approximately 1.5 years depending of the size of the stocked salmon and what season it was stocked. Before the salmon is transported for slaughtered it is starved for 7-14 days depending on water temperature. The starvation has its purpose to increase tenacity and reduce the possibility of transportation and handling induced stress. It is thought that stress generates a substance enhancing bacteria growth. Which may lead to a poor finished product, smell, taste and texture wise.

At the processing facility the salmon is sedated, killed, gutted, washed, sorted after size and quality and put on ice. It is rare that the processed salmon is further processed into fillets or other fish products in Norway. The exported salmon usually is whole gutted salmon. After the salmon has been collected from the fish cage to slaughter, the fish cage is fallowed. The fallowing time depends on various factors as sea currents and sea bottom sediment values. Normally fallowing is lasts for two months. In this period the fish cage is maintained often put onshore to remove algae growth on the nets, investigate for weaknesses in the net and repairs. After the fallowing period the production process starts over. The production process from hatchery to slaughter can be seen illustrated in figure 1

1.3 Salmon louse life cycle

There are two species of salmon louse, Lepeophteirus salmonis and Caligus elongatus von Nordmann, which is found on salmon in the atlantic ocean. Of these two the Lepeophteirus salmonis has caused large losses among the salmon producers located in the north-eastern parts of the atlantic ocean. As Lepeophteirus salmonis is the one causing the large losses this salmon louse will be investigated. Any referral to salmon louse or lice in the thesis is therefore to Lepeophteirus salmonis, L.salmonis. The salmon louse (Lepeophteirus salmonis) is a sea louse, a parasite mainly living on salmonoids. It is a species of copepod in the genus Lepeophteirus. It has a direct life-cycle (i.e. a single host) with eight life stages and each life stage is separated by a moult. The Lepeophtheirus salmonis is on an average twice the size of most Caligus specieses. The body of an adult L.salmonis consists of four regions: abdomen, leg-bearing segment, cephalothorax and genital complex. The cephalothorax acts like a suction cup where it holds itself on the fish. It forms a broad shield that includes all body segments up to the third leg-bearing. It has a mouth part shaped as a siphon. In addition to the cephalothorax, second antennae and oral appendages are modified to assist the louse in holding the louse on the fish. The second pair of antenna is also utilized by adult males to grasp the adult female during copolation.

The salmon louse is not a parasite that multiplies on the host, which is essential in understanding the salmon louse problem. The salmon lice in one farm is a consequence of a generated copepodid pressure from salmon lice located elsewhere. Even for a large production site it is unlikely for one cage to infect another within the site. This is due to the earliest stages of the salmon louse where it drifts with current and is non-parasitic for minimum 2 days at temperatures aorund 15 °C.

It has been believed that L.salmonis does not reproduce or grow during the winter. However, studies conducted at temperatures down to 2 °C has proven that eggs develop. It took 45.1 days and a large portion of the nauplii developed into copepodid down to temperatures of 4 °C (Boxaspen & Naess, 2000). Which disproves this statement.

1.3.1 Nauplii 1 & 2

The salmon louse hatches directly into the water as a planktonic nauplii. These nauplii are non-feeding and not infectious until they molt into copepodid. Nauplii 1 is very salinity responsive, and newly hatced nauplii 1 does not survive at salinities of 15% or less, and poor development at salinities between 20% and 25%. The time spent in nauplii stage 1 and 2 is estimated to be approximately 45 hours at water temperatures of 15 Celsius and 222 hours at 5 Celsius (Tully & Whelan, 1993). Theoretically in the winter season the nauplii can be transported 279 km at sea currents of 0.35[m/s] before molting into the infectious copepodid stage. Both the nauplii and copepodid stages are positively phototactic and has a daily vertical migration, sinking at night and rising at day.

The nauplii is part of the plankton feed chain and is by that equally susceptible to predation. It is estimated that 70% of all zoo-plankton is subject to predation, and there are no reasons why nauplii 1 & 2 should not suffer same losses. It is also found that they are susceptible to bacterial infections, virus and parasites, their effects are little known, but they tend to increase with temperatures rising above 15 °C

1.3.2 Copepodid

In this thesis copepodid refers to the infectious copepodid stage between Nauplius 2 and Chalimus 1. Other papers may refer to copepod for this stage, however copepod may be confused with the meaning of any very small crustaceans of the subclass copepoda. And therefore is being used, however literature widely uses copepodite as any of the five stages in the life-cycle of a copepod prior to the sexually mature adult. This should be in mind when searching for additional information regarding the subject.

The copepodid actively search for a host through sensing vibrations in the water, and their ability to find host is not light dependent. Heuch, Parsons, and Boxaspen (1995) proved that copepodid swim upwards in response to pressure and a change in water flow or a mechanical vibration, which induced a burst in swimming speed. This is backed by (Heuch & Karlsen, 1997) Heuch and Karlsen (1997) whom reported that copepodid are sensitive to low frequency water accelerations, such as for example produced by a swimming fish. When a host is found thecopepodid clasps the host tissue, then undergoes a molt to the first sessile stage in the life cycle if it decides to stay on the host. It then uses its claws to penetrate the salmons skin, and a form of glue is released into the salmon forming a more permanent attachment(Mortensen & Skjelvareid, 2015). The salmon is now infected by salmon louse and the copepodid will molt further until it becomes mature able to reproduce. After initially attachment of copepodid, the process to final stage takes approximately 52 days for male and 57 days for females at water temperature of 10 °C. The development of the copepodid is strongly correlated to water temperature as for the other stages as well. Samsing et al. (2016-) has stated a formula for estimated days from hatched to infectious copepodid and the estimated amount of days of infectiousness of salmon louse as a function of temperature. Which will mean after how many days does it take for a newly hatched nauplii 1 to develop to a copepodid, and how long does it stay in this stage before it eventually dies/finds a host. The functions has been plotted and can be seen in 2 They also found that below 5 °C there were not much infection at all.

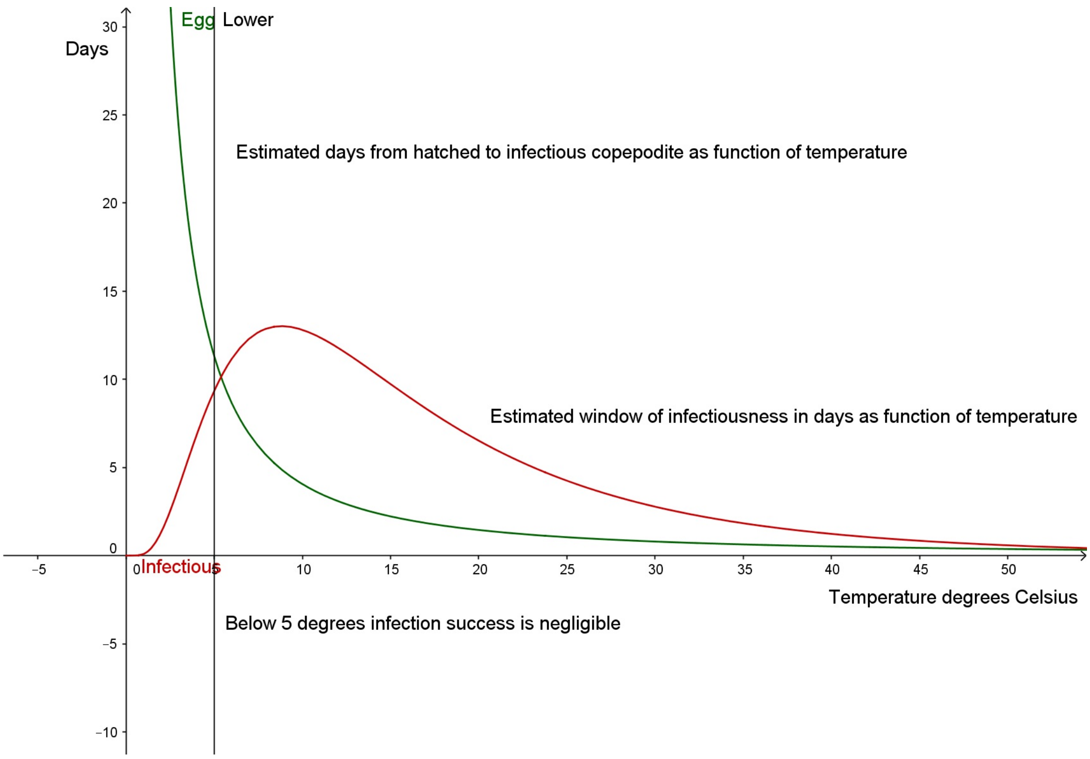


Figure 2: Development rate of infectious copepodites

$$\text{Days from hatched to infectious} = e^{1.4-1.48*ln\frac{T}{10}} \tag{1}$$

$$\text{Duration of infectious stage} = e^{2.55-0.26*ln\frac{T}{10}-1.03(ln\frac{T}{10})^2} \tag{2}$$

Where T is the seatemperature. The functions are plotted in figure 2

When it comes to the nauplii and copepodid movement, Heuch et al. (1995) found that copepodid displayed a distinct diel vertical migration pattern. Which means that they gathered near the surface during the day time, and at night spread out into deeper layers of the water. However, nauplii only showed small differences in day and night depth, while the copepodid distribution seems to follow this pattern consequently. This pattern is opposite of the wild salmon diel vertical migration pattern, which may prevent many copepodid in finding a host in the wild. This again poses a threat to the farmed salmon as they are fed during the day, which means that they are near the surface simultaneously with the infectious copepodid. And not fed during the night which makes the salmon go deeper matching the pattern also during nigh-time. Infection vice Heuch and Karlsen (1997) observed copepodid being able to generate a short-term burst swimming speed of 9 cm/sec for 1 second, and after the burst a more prolonged speed of 2 cm/sec when stimulated to swim. Which indicates that the copepodid may drift until it senses a salmon for then bursting to infect the salmon. How it distinguish salmon from other fish is uncertain, it is however thought that the copepodid when clasped to the fish identifies its host. This however would consume unnecessary energy, and it is more likely that it detects that the fish is a Salmonidae before it bursts and attaches to the host. In bath tests, it has been proven that the majority of copepodid chose to settle on salmon rather than cod. And four hours post-infection copepodid chose to change host from cod to salmon post settlement (Pert, Modue(Luntz), Fryer, O'Shea, & Bricknell, 2009). It was also seen that the salmon louse did infect other species such as cod, but after 24 to 28 hours post infection, the numbers were drastically reduced. And after 96 hours no salmon louse was left on the cod. Indicating that the cod is a unsuitable host for the copepodid(Pert et al., 2009). Temperature has also been proven to affect the infectiousness of the copepodid, Tucker, Sommerville, and Wootten (2000) showed that a larger proportion of copepodid failed to establish themselves on a host at 7°C than at 12°C.

1.3.3 Chalimus

Chalimus stage consists of two stages chalimus 1 and chalimus 2. At this stage the louse is sessile meaning it is attached to the salmonoid throughout these two moults before becoming a pre-adult mobile stage. The first chalimus stage has well-developed mouthparts and a functional alimentary canal, and is the first feeding stage in the life cycle (Bron, Sommerville, & Rae, 1993)

1.3.4 Pre-adult

Shortly after moulting from chalimus stage the pre-adult L.salmonis has the ability to detach from a temporary frontal filament and move over the surface of the hos. It is first in the pre-adult stages one can distinguish male from female. The genital complex is under-developed and the mean length is about 3.6mm. Final moults to adult stages, both male and female, then take place. The female is larger than the male with males measuring 5-6mm and females 8-18mm.

1.3.5 Adult

When counting salmon lice at each fish farm location, it is the adult females that is being the standard of measurement. Mainly due to the louse limit set as adult female pr salmon average, as the adult female is a measure of ongoing reproduction of salmon louse. There has been discussion whether to count only fertilized females or not, all females regardless of whether they are fertilized or not shall be counted as adult females can develop egg strings within a few hours/days. The adult females are significantly larger than the adult males and their genital complex is the same length as their whole body, and darkens upon maturation. The adult female L. salmonis can produce ten to eleven pairs of egg strings over their life cycle of approximately seven months, with a mean egg number per string of 152 at 7.2 °C (Heuch, Nordhagen, & Schram, 2000) and around 500-700 at higher temperatures. Females have been found in laboratory experiments to carry eggs after 15.5 months (Hamre, Glover, & Nilsen, 2009). The numbers of eggs per string varies a lot among the adult females, however higher temperature generally results in higher numbers of eggs. The eggs darken upon maturation.

The pre-adult, adult and especially fertilized adult females, are aggressive feeders. Aggressive as in they some times feed on blood in addition to tissue and mucus. Blood can even be seen in the digestive tract, easily seen for fertilized females. When feeding they secrete trypsin into their host's mucus. Trypsin is a serine protease which hydrolyses proteins, making it easier for the L.salmonis to feed and digest, and may even help it avoid the immune response of its host.

1.3.6 Fecundity

Fecundity is the reproductive rate of an organism or population, in this case organism as a measurement of number of eggs. Which has been proven to vary from 0 to 1000 eggs per set of egg strings (Jackson & Michin, 1992) . The bigger the adult female, the higher number of eggs is produced per set of egg strings. Number of eggs are also temperature dependant, and the number of pair of egg strings produced per time increases with temperature (Heuch et al., 2000). The salmon louse has highest fecundity at temperatures around 7-15°C and lower at temperatures over 20 °C and around 3 °C. It is known that one copolation is sufficient to fertilize 11 pair of egg strings at the same adult female. The production of egg strings are continuous, and after hatching it starts producing new eggs. At 10 °C adult females produce a new pair of egg strings every 10th day. And even faster at higher temperatures. Meaning one copulation may lead to 11 pair of egg strings hatching 11*(0−1000) new L.salmonis nauplii after only 110 days at water temperatures around 10 °C . The whole life cycle with all stages can be seen in figure 3

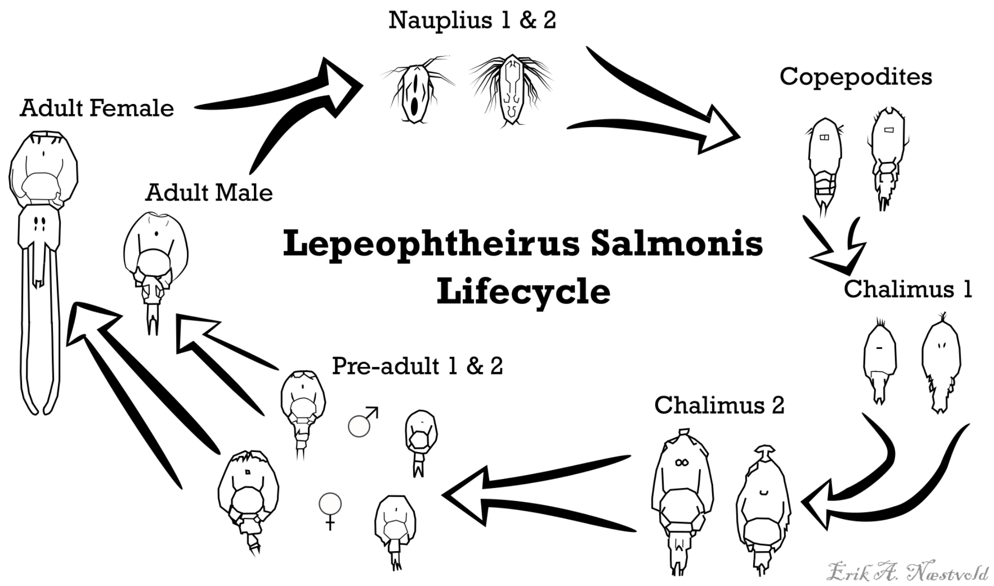


Figure 3: Life cycle of Lepeophteirus salmonis

Stage	Temperature for duration [°C]	Duration [hours]	Infectious	Tolerance for reduced salinity
Nauplius 1	[5,10,15]	[52,19,9]	No	Low
Nauplius 2	[5,10,15]	[170,68,36][3]	No	Low
Copepodid	[5,10,15]	[240,192,48][1]	Yes	Low
Chalimus 1	[10]	[360]	Sessile	NA
Chalimus 2	[10]	[240]	Sessile	NA
Preadult 1 female	[10]	[240]	Mobile stage	Good
Preadult 1 male	[10]	[192]	Mobile stage	Good
Preadult 2 female	[10]	[288]	Mobile stage	Good
Preadult 2 male	[10]	[192]	Mobile stage	Good
Adult female	[]	15.5 months[2]	Mobile stage	Reduced hatching success. Survivability: good
Adult male	[]	[NaN]	Mobile stage	Good
Fertilized eggs	[5,10,15]	[419,207,130]	No	Very low. development vice

Table 1: Development rate of L.salmonis stages

*1 Copepodid have stayed in this stage for 1 month in laboratory, in wild not confirmed.

*2 There are little to no evidence as of how long an adult female will survive on average. However, (Hamre et al., 2009) found three females carrying eggs after 15.5 months.

*3 Tully and Whelan (1993) summarized data describing the length of time that L. salmonis remains in the noninfective nauplius I and II stages as varying between 223.3 h at 5°C; 87.4 hours at 10°C; and 50.0 hours at 15°C.

2 System description

The aquaculture of atlantic salmon is the farming and harvesting of atlantic salmon under controlled conditions for mainly commercial purposes. Atlantic salmon is the most valuable fish group for Norway measured in net value. The production of atlantic salmon can generally be divided into two stages. First stage includes the hatcheries where they are hatched and raised on land in freshwater tanks. When they are 12 to 17 months and has become smolt, they are transferred to floating sea cages for the second stage. This production stage is usually in a marine environment, and is known as mariculture. When reached desired size 4.5 – 9 kg, they are transferred from the sea cage to the processing plant by wet well ships. Where they are killed, bled and iced.

A production site further noted as fish farm, does consist of one or more fish cages depending on their allowed volume of bio-mass to produce. Feed barge which supply each cage with food stored on-board the barge, and mooring system which holds the fish farm attached to designated location. One of the governing aspects of the production area is fish welfare. There are several aspects to be aware ranging from virus and bacterial related diseases, to predators and salmon louse. The salmon louse problem is of particular interest as of date due to the environmental threat it poses. The magnitude of production salmon in the sea has been capped as the Norwegian does not issue new regular salmon licenses before the problem has been handled sufficiently. Which drastically reduces the possibility for further growth in the industry. A license gives a right to have maximum 780 tonnes of salmon in the ocean at any given time. It is usual for a farm site to hold more than one license, some of the big sites has up to eight licenses. The only licenses that may be issued per date are so called green licenses, which yield extra terms to be followed. These terms may be a lover sea louse average per fish compared to regular licenses, or other environmental measures like using closed cages instead of regular net pens.

Salmon louse is not a new problem, and has been present since the beginning of Norwegian salmon mariculture. The salmon louse, L. salmonis, hatches directly into the water as a planktonic nauplii. These nauplii are not infectious until they molt into copepodites. The time spent in nauplii stage 1 and two is estimated to be approximately 45 hours at water temperatures of 15 Celsius and 222 hours at 5 Celsius. Theoretically in the winter season the nauplii can be transported 279 km at sea currents of 0.35[m/s] before molting into the infectious copepodites stage. At this stage it has the capability of moving both horizontally and vertically, and is searching for a host. It is thought to detect its host through fish movement generating shock waves, which the copepodites swims towards. However, the L. salmonis is species specified so it will not attach to a host outside the salmon family. When a host is found the copepod clasps the host tissue, then undergoes a molt to the first sessile stage in the life cycle. The salmon is now infected by salmon louse and the copoepodite will molt further until it becomes mature able to reproduce. Duration from initially attachment for copepodites to reproductive adult takes approximately 52 days for male and 57 days for females at water temperature of 10 Celcius. The adult female L. salmonis can produce ten to eleven pairs of egg strings over their life cycle, with a mean egg number per string of 152 at 7.2 Celsius (Heuch et al. 2000.) And the cycle continuous as long as there are hosts to be found, which there are abundant of in fish farms. There are 943 fish farm sites along the Norwegian coast, where 941 of these are exposed to L.salmonis. Each fish farm contains from one to 16 fish cages, each cage containing up to 200 000 salmon. There are roughly speaking 260 million caged salmon evenly spread across the Norwegian cost, 260 million potential hosts for the copepodites. Evenly spread as these farm sites must be placed at sheltered locations, leaving few distances along the coast untouched. As close to all of these cages utilize open net-pens, leaves infection bound to happen.

Several measures has been developed to combat the spread and abundance of salmon louse. These delousing measures can be divided into two different fields, active and passive delousing methods. Passive delousing methods are measures which constantly are removing salmon, preventing salmon louse growth or hindering copepodites from entering the cages. The active methods are often more comprehensive as they aim to remove all louse present in a fish cage during a short period of time. The common procedure as of today is implementing passive delousing methods, and in an event of violating the average salmon louse per salmon limit, an active method is utilized.

There are several different passive delousing methods to hinder the rise of a salmon louse population in cages. The most known methods are cleaner fish, cage skirts and laser removal These methods are classified as passive as they are implemented in the fish cages and are continuously preventing or removing salmon louse. However, when the densities which violates the given maximum number of louse per fish, which most often in Norway is from 0.2-0.5 adult female per salmon, measures must be taken. This is when the active delousing methods come to part, this paper will mainly focus on these active methods.

As of the active delousing methods it is convenient to separate these from each other as they vary a lot over ideology, needed resources and procedures. These methods are bath treatment, feed and mechanical. Bath treatment utilizes a variety of chemicals which exterminate the salmon louse at direct contact. Feed is food mixed with medicine fed to the salmon which in turn affects their blood stream and therefore the attached salmon louse. Mechanical utilizes equipment which physically removes the salmon louse, as of such brushes and high pressure water. Temperature and fresh water are also used as means of delousing.

The system described in this thesis can generally be divided into two parts, the salmon louse with its behavior and biology, and what measures are at hand to combat it. However, it is the interaction between these two systems which is of interest. The focus will be on investigating this system, and on basis of the findings, develop a regional delousing strategy.

3 Problem description

As the increase of salmon in mariculture over the last decades, the salmon louse presence has increased proportionally if not more. The wild stocks of salmon has throughout our knowledge of it, yielded the L.salmonis. The densities of L.salmon on wild salmon is often recorded with numbers way above the set limits in fish farms. The salmon louse does decrease salmon growth and is therefore unfavourable addition in the fish farms. However, grown salmon does not die and perish as a result of above 0.5 salmon louse per salmon, as large wild salmon has been caught with numbers exceeding 60 louse. Therefore, it is illogical to see the set limits of 0.5 and 0.2 as a contribution to the salmon mariculture. Rather a 2.500 billion NOK direct delousing cost per year and hundred thousand delousing related salmon deaths disadvantage. What is however the reason for this set limit and a logical as such is the large and dense copepodite pressure areas the infected fish farms induce over the coast. These areas not only infect other fish farms but affect the wild stocks. Grown adult salmon are much less susceptible to salmon louse compared to the wild smolt. This induced copepodite pressure is almost solely fish farm induced and oppose a large threat to the new wild stock generations, where smolt dies from unnatural high salmon louse infectations. As will be discussed the salmon louse attached to one salmon does not result in more salmon louse on the same salmon, the salmon louse is a direct consequence of external copepodites produced from a different salmon. Mainly due to the non-infective stage of newly hatched nauplii which are not capable of movement

The only per date measure to reduce the induced copepodite pressure is the salmon louse density limit. A new system regarding collective punishment for areas exceeding a limit of shared salmon louse abundance is out for hearing at the moment. Which makes sense as it is the copepodites pressure which is of importance, and certain farms and areas may expose a larger threat towards the wild smolt than other locations. Locations such as fjords and coast near important wild salmon rivers. Regarding the density limit it is up to each farm site to collect and count salmon louse each week and undertake measures to keep the densities under this limit. This is achieved by implementing passive delousing methods and occasionally active delousing methods when the limit has been violated. This can be viewed as merely maintaining the problem rather than solving the overall salmon louse problem. As the source of salmon louse through the copepodites pressure is still present regardless of the amount of salmon louse present at the location. Only by removing the copepodites pressure and present salmon louse, one can expect a salmon louse free fish farm. This can only be achieved by a regional delousing plan, where the dominant sources of induced copepodites pressures are located and handled. Together with a plan for the active delousing methods preventing fish farms located in copepodites pressure locations delouse before the farm inducing the pressure is deloused. However, as the whole Norwegian coast is packed with salmon farms this means that planning a delousing in Northern Norway is relying on pressures generated all along the coast down to southern Norway. Some fish farms may be located in such manner that they work as relay stations from one area to the next, through their in and out-flux of copepodites. Identifying these governing fish farms with and treated them accordingly would be of importance in developing a sustainable delousing regime.

Where active and passive delousing measures are their tools in achieving sustainability.

In order for this planned regional delousing regime to come to part, there is a need to understand the equipment at hand. As of today the means of delousing is through implementation of passive/preventive delousing methods, and contact an active delousing company they know of in the event of exceeding the abundance limit. However, these active methods have their limits and applicability. Which favour some measures for the specific conditions related to each farm. These conditions are typically environmental conditions as water quality, weather and temperature. Increasing resistance towards the different measures has also been recorded along areas of the coast, which renders the efficacy of the affected measures. There have been several incidents of mass salmon death in relation to delousing operations. Which may come from the lack of knowledge regarding each delousing agent's applicability and constraints. There are several papers published regarding these aspects however not as readily for the common man to grasp. The problem to be solved in this thesis is the question regarding choice of delousing method, and when. The thesis will explain and display all current active and passive/preventive delousing measures available in Norway, with their constraints and applicability. The thesis should be of interest for everyone interested in the mechanisms of the L-salmonis bluntly put, and the variety of methods available for removal and prevention of the salmon louse. The intention of this paper is to give a thorough yet readily understandable overview and explanation regarding the delousing measures and their respective delousing agent/compounds/usage. In order for each salmon farm to make optimal decisions and long term preventive cycle to control the salmon louse epidemic. Further problem to be solved is how to exploit the findings in developing a regional delousing system. This has been develop through a simulation model which aims to simulate the region with fish farms, environmental aspects and present copepodite pressures. Estimating the results of implementing delousing measures at each individual fish farm on a regional scale.

Implementing the simulation results in a region faces a series of problems. First of all, there are rarely any larger regions controlled by one fish farm company, leading to one not cooperative company jeopardising the whole plan. Second is the need for increased screening of salmon louse abundance in each farm, in order to perceive the situation correctly. And not be deceived by under reporting of the amounts of salmon louse. Third is the availability of the resources required to execute the measure at desired time. The salmon louse has an tendency to rise above the limits during the fall, leading to scarce available resources. Last is the validity of the variables forming the simulation model. As there are several aspects described through the variables which are of great uncertainty.

4 Active delousing methods

Delousing is the process of implementing/executing a measure which has as intention to hinder/remove salmon louse abundances at fish farms. There are many measures available and has been categorized after usage. The main categories of delousing can be said to be active and passive delousing methods.

The active methods are often comprehensive operations which aim to remove/decrease salmon abundances over a period of time. The operation varies from the different active delousing methods. The most common treatment procedure is related to topical treatment by use of wet well boats or tarpaulin. Recent years, there has been an increase in usage of mechanical measures, which does not utilize tarpaulin or wet well boats rather external instruments treating each individual fish. Both mechanical and bath treatment are means of topical delousing. The third and last category of active delousing methods are oral treatment. It differs from the topical treatments in both time duration often ranging over a week and has the benefit of not requiring any handling of the fish related to the treatment. Fish handling has been thought to cause stress for the salmon. Handling such as crowding, pumping and general maneuvering, which is necessary when treating the salmon topically.

4.1 Bath treatment

Topical/bath treatment is the most common way in delousing salmon with chemicals/drugs by exposing the salmon louse to a delousing agent through direct contact. The method relies on confining the population to be treated in a known volume of water of which a delousing agent is added to expose the fish to the desired concentration. The closed volume containing the louse infected salmon is either created by transferring the salmon into the well of a well boat, or by penetrating their respective cage and enclose it by a tarpaulin. The enclosed volume should be known as this determines the quantity of delousing agent to be applied. Determining enclosed volume for penetrated cages with tarpaulin is not as readily as for a well boat. Therefore, some delousing agents should only be used in combination with well boats.

As the delousing agent is costly and often toxic to the environments one wish to use as small quantity of delousing agent as possible, but enough to treat the salmon properly. Therefore, the treatment volume should be as little as possible, but large enough for the salmon to thrive. The volume must be closed, therefore bath treatment occurs in well boats or by applying a closed tarpaulin around a penetrated cage. We will focus on closed bath treatment by tarpaulin as the two operations are equal in manner but deviates in equipment and enclosed volume.

Weather conditions play an important role in tarpaulin based bath treatments. The tarpaulin is easily deformed by currents, and the practical aspect of handling is heavily weather dependant relying on calm conditions. It is not uncommon to abort a tarpaulin treatment before deemed finished as the weather may make the process unsafe to continue, for both fish and personnel.

In table 2 one can see the different chemical/drug delousing agents used in bath and oral

Delousing method	Classification	Active substance	Known trade names
Bath treatment	Pyretroids	Low-cis cypermetrin	Excis vet.
		High-cis cypermetrin	Betamax®
		Deltametrin	Alpha Max®
		Pyretrumextract	Py-sal vet
	Organic phosphorus compounds	Azamethiphos	Salmosan®
	Antiseptics	Hydrogen peroxide	Paramove®
Food supplement	Avermectins	Emamectin benzoate	Slice® vet.
	Chitin synthesis inhibitors	Diflubenzuron	Lepsidon vet.
		Teflubenzuron	Ektobann

Table 2: Categories of delousing agents used in bath treatment and through food supplements, often referred to as chemical delousing

treatment. This section will focus on the different medicines used in relation to bath and oral treatment of salmon louse.

4.1.1 Operation procedure

Topical delousing is often done through bath treatment. The bath is generally achieved through use of tarpaulin or the wells of wet well boats. The enclosed volume should be known as this determines the quantity of delousing agent to be applied. Determining enclosed volume for penetrated cages with tarpaulin is not as readily as for a well boat. Therefore, some delousing agents should only be used in combination with well boats. The operation can be divided into three parts: Initialization, execution and de-mounting and completion. An illustration of mounting the tarpaulin on a penetrated fish cage can be seen in figure 4.

It is important to have an idea of the salmon's current health status, especially regarding heart- and gill health before delousing. As the delousing process is posing stress onto the salmon, and with a poor health status may and has many times lead to loss of salmon. The salmon must also have been sufficiently starved, approximately between 2-4 days is recommended. The starvation is to increase tenacity of the salmon prior to the treatment. One of the reasons is the reduced oxygen requirement when the fish has no food to process.

See appendix... for an in-depth explanation of the procedure of bath treatment with tarpaulin.

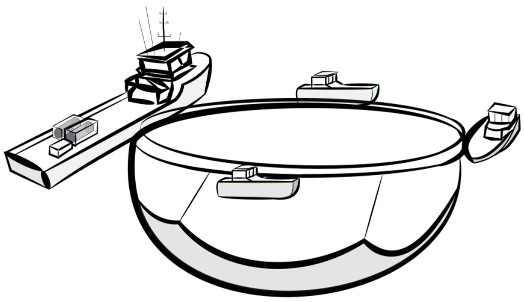


Figure 4: Mounting of tarpaulin on penetrated fish cage

4.1.2 Determining enclosed volume

For both bath treatment in enclosed cage by tarpaulin and in the well of a wet well boat it is essential to know the amount of delousing agent to add to the bath in order to achieve desired concentration. This is achieved by knowing the enclosed bath volume, and add delousing agent accordingly. However the enclosed volume is somewhat unknown as of bath treatment by tarpaulin and uncertain system vice for delousing in wet well boats.

Most wet well boats has an accurate estimate of the enclosed volume of their wells. However it is usual to calibrate the well when it is supposed to use hydrogen peroxide as delousing agent. This calibration is only necessary to do once to check the functionality of the whole system. System as of well, pipes connected and dispersion of delousing agent. The piping and sensors should be on point in order not to apply too much/little hydrogen peroxide killing or not affecting the salmon in the well.

For bath treatment in cage by tarpaulin one does not know the volume. Which makes it essential to figure the enclosed volume before adding delousing agent. For hydrogen peroxide the method is pretty much equal to the one in well boats. One make an estimated guess of the enclosed volume, add an amount of hydrogen peroxide then measure the concentration in the enclosed volume this amount of hydrogen peroxide generates. As one know the amount of hydrogen peroxide added, and the resulting concentration, it is readily to calculate the volume. However for the other delousing agents this is not necessarily the case. The tarpaulin can be of much help when estimating the enclosed volume. By adding diameter marks on the tarpaulin and apply it accordingly, one can read off what diameter of tarpaulin has been used to the enclosure and determine tarpaulin surface area. As one know the circumference of the cage and surface area of the enclosed volume. One can by use of the implicit formula of surface area of a oblate or prolate sphere iterate the according enclosure depth. And thereby estimate the enclosed volume. See appendix F for a Matlab function determining enclosed volume from cage circumference and read tarpaulin diameter/circumference (enclosed volume = function(Tarpaulin circumference, Cage circumference). It should be noted that this function estimates enclosed volume on background of the assumption that the tarpaulin is perfectly applied without any slack. And should therefore only be viewed as an estimate.

4.1.3 Pyrethroids

Pyrethroids are synthetic insecticides based on natural pyrethrum which constitute to a large amount of commercial household insecticides. The toxic effect is through preventing the closure of the voltage-gated sodium channels in the axonal membranes («Pyrethroid,» 2016), which left open permanently makes the axonal membrane depolarized which paralyzes the organism affected. Bluntly put cypermethrin and deltametrin are neuorpoisons acting on the central nervous system in mammals and insects.

The pyrethroids used in delousing are synthetic pyrethroids and the three most known synthetic pyrethroids used are: Deltametrin, High and low-cis cypermethrin. More known in mariculture by their trade names: Deltametrin - (Alphamax®,Pharmaq) , Low-cis cypermetrin - (Excis®, Novartis) and high-cis cypermetrin - (Betamax®, Novartis). All three synthetic pyrethroids are authorised in Norway and Faeroes for use against salmon louse, and has been widely used. They have been proven to be 95-99% effective against mobile stages of L.salmonis, and approximately 85% on all stages before pre-adult. However, these efficiencies does not account for the increased resistance developed. Used today the efficiency has proven to be varying alot from one delousing to the next.

Pyrethroids is not temperature dependant and can generally be used all year round, but not under 6ºC (Rykhus et al, 2012). However, due to the temperature limitations of other delousing agents/methods (see heat treatment). It is recommended only to use pyrethroids at high water temperatures 15ºC+ as other delousing agents/methods should not be utilized at these temperatures.

4.1.4 Organic phosphorus compounds, azamethiphos

Organic phosphorus compounds are as the name depicts, organic compounds containing phosphorus. They are primarily used in pest control as an alternative to chlorinated hydrocarbons that persist in the environment. Which is of great importance as the bath treatment water often is poured untreated back into the sea.

Azemethiphos is an organophosphorus insecticide which in veterinary medicine is used in fish farming control of salmon louse. It is stated that azamethiphos had a very low margin of safety in treatment of Atlantic salmon. Therefore correct dose level is of utmost highest importance. Over-dosing can easily result in death for Atlantic salmon (EMEA, 1999). This report also concluded that within 12 hours after treatment of salmon louse with azemethiphos, residues of azemethiphos were below the limit of detection. Meaning that the azemethiphos obtained by the salmon after treatment disappears quickly and are of no concern regarding human consumption. Azemethiphos should be used with care at water temperatures above 10 ºC and preferably never above 15 ºC due to toxticity to the salmon. It is known to be efficient towards pre-adult and adult stages of the L.salmonis, and not so much towards earlier stages. Which makes it unsuitable in case of a dense presence of copepodite pressure. however suitable near winter/winter when the temperatures and L.salmonis development rates are low.

Most commonly known organic phosphorus compound used in delusing is azamethiphos under the trade name Salmosan®. It is known to be only effective against pre-adult and adult stages of L.salmonis, with excellent efficacy towards adult females (Horsberg T, 2011) The treatment time varies from 30 to 60 min depending on time. It is authorised in Scotland, Norway, the Faeroes, Chile and Canada, and has been widely used in these countries. However increasing resistance towards azamethiphos has reduced the usage of the organic phosphorus compound.

I 2007 ble middelet i denne gruppen, Salmosan, tatt helt ut av markedet. Det hadde ikke lenger særlig effekt. (Tor Einar Horsberg) http://www.bt.no/nyheter/lokalt/Jobber-med-alternative-mottrekk-for-a-stoppe-lakselusen-299399b.html

4.1.5 Deltametrin and azamethiphos resistance

A study was undertaken by (Sevatdal, 2014) to observe and investigate the development of resistance in laboratory designed L.salmonis with resistance markers to azamethiphos (Salmosan®) and deltametrin (AlphaMax®). The L.salmonis was categorized as of their susceptibility to each of the delousing agents. L.salmonis sensitive to deltametrin and with resistance towards it, and low, medium and high resistance towards azamethiphos. With these five categories of L.salmonis, several treatments with deltametrin and azamethiphos was undertaken. Both with single agent treatments and combination of the two. The L.salmonis prone to the treatment where all adult.

The study showed that treatment with azamethiphos, deltamethrin and in combination are very effective against L.salmonis with no resistance towards the delousing agents, so called sensitive L.salmonis. It was shown that the genotypes promoting resistance altered after treatment. Demonstrating the treatments with these agents are an active driving force towards higher resistance.

The delousing agents showed almost no effect towards higly resistant louse. Azamethiphos killed approxemately 95% of the L.salmonis without resistance genotypes, 85% of the medium level of resistance but only 9% of the L.salmonis with high resistance genotypes. Deltamethrin killed allmost 100% of the L.salmonis with no resistance genotypes after 1400 minutes but only 8% of the L.salmonis with resistance genotypes. The combination treatments proved to not be the most efficient against L.salmonis with no resistance genotyopes compared to single agent treatment. However, for Deltamethrin resistant L.salmonis combination treatment was the most effective with 60% of the L.salmonis killed. For Azemethiphos resistant L.salmonis sole Azamethiphos treatment was most effective with barely 10% of the L.salmonis killed. Which would imply that Azemethiphos is effective against Deltamethrin resistant L.salmonis but Deltametrin has close to no effect on Azemethiphos resistant L.salmonis.

The surviving L.salmonis from the various treatments were later treated with hydrogen peroxide. A concentration of 1300 mg/L was used and resulted on an average efficacy of 42%. Where the efficacy on the control group with no resistance was 55%. Which show little correlation between Azemethiphos and deltametrin resistance and eficacy towards hydrogen peroxide. What should be noted is that a hydrogen peroxide concentration of 1300 mg/L is much lower than 2200 mg/L which is the standard of Hydrogen peroxide treatment per date(Questions, Aqua Pharma).

The large gaps in treatment efficancy (% of L.salmonis killed after treatment) implies the importance of knowing what genotypes the L.salmonis has before treating a fish cage with azemethiphos and especially deltametrin. Not only can the fish cage treatment achieve a low efficacy and require a new treatment but most likely aggravate the resistance towards the delousing agents.

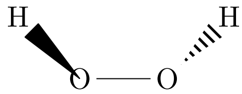
4.1.6 Antiseptics

Antiseptics are antimicrobial substances usually applied on living tissue/skin to reduce the possibility of infection, sepsis, or putrefaction. They differ from antibiotics as they do not yield the ability to be transported thorugh the lymphatic system to work within the body. And differ from disinfectant which destroy microorganisms found on non-living objects. Antiseptics are widely used in wound treatment on humans and animals.

A well known antiseptic is hydrogen peroxide. Hydrogen peroxide is a highly oxidizing compound, it does also have reducing properties depending on pH. In high concentrations, hydrogen peroxide is an aggressive oxidizer and will corrode many materials, including human skin. In the presence of a reducing agent, high concentrations of H_2O_2 will react violently. To visualize the potency of hydrogen peroxide, high concentrations of H_2O_2 are in combination with catalysts used as rocket propellants. Hydrogen peroxide has the significant beneficial trait of decomposing into H_2O and O2 by itself, which means that the decomposed product is of no harm to the environment. Regardless of this fact, treatment with hydrogen peroxide has been blamed for several incidents of negative environmental impacts.

Hydrogen peroxide is thermodynamically unstable and decomposes to water and oxygen $2\,H_2O_2 \xrightarrow{\Delta h_1} 2\,H_2O^+ + O_2 + \Delta h_2$. The rate of decomposition increases with rising temperature, concentration of H_2O_2 and pH, with cool, dilute, acidic solutions showing the best stability. Where Δh_1 is required energy to decompose and Δh_2 is released energy.

Branched Hydrogen peroxide molecule:



The toxicity of hydrogen peroxide towards sea louse is not clearly understood, it is suggested that H2O2 causes a mechanical paralysis as a result of decomposition of H_2O_2 to O_2 and H_2O in the gut of the salmon louse (Grant, 2002). Based on the fact that there are L.salmonis present at the surface seemingly unresponsive to stimulation. Recovery to normal movement after paralysis has been noted experimentally (Hodneland, Nylund, Nilsen, & Midttun, 1993). The author has been present and done some research of his own on this matter. After releasing the tarpaulin a ROV was monitoring the fish behaviour and it was clear that the fish was distressed and was grouping up towards of the bottom of the net pen, most likely as a natural salmon behavior in case of distress or as I believe, to escape highly toxic solutions formed from hydrogen peroxide in contact with the newly introduced sea water. Hydrogen peroxide in contact with metals as iron and copper form higlhy toxic gas boubles, which may be the reason for the salmon behaviour. Regarding the toxicity towards L.salmonis, I believe that there are two reaction patterns towards hydrogen peroxide. As hydrogen peroxide is an aggressive oxidizer and will corrode many materials, including human skin. The blunt tought is that for a small organism as the L.salmonis compared to salmon salar, L.salmonis is oxidized to death upon exposure. And in case of high values of catalase within the L.salmonis the hydrogen peroxide is decomposed before it is oxidized sufficiently. This again generates oxygen within the L.salmonis. This hypothesis is confirmed by visual confirmation of large quantities of L.salmonis sinking through the net pen after exposure, and grouping of L.salmonis at the surface. Bubble wrap was applied on the surface of the fish cages after the hydrogen peroxide treatment, resulting in large concentrations of adult L.salmonis attached to the bubbles. However these numbers can not account for the total population of L.salmonis prior to the treatment. The fact that H_2O_2 has a great efficacy towards nauplii 1 & 2 and L.salmonis eggs promotes the theory regarding oxidizing as the toxicity of H_2O_2 (Rykhus et al, 2012) .

Applicability of hydrogen peroxide has been stated to be constrained by water temperature. (Thomassen, 1993) experimented on the use of hydrogen peroxide on salmon and observed gill damagage described as "sloughing", 80% of the salmon with "sloughing" which was treated at temperatures of 14°C and 18°C were dead after 24 hours. Another study performed by (Kiemer, 1997) investigated the correlation between temperature and mortality related to delousing with hydrogen peroxide. Concentrations of 1370, 1460, 1720 and 2580 mg^{-L}/ppm were used at temperatures of 10, 13.9, 14.5 and 16°C. Mortality was negligible at temperatures ranging between 10 and 14.5°C. However, a 100% mortality was the result of 2580 mg^{-L} at 16°C, within 24 hours. At all combinations gill damage was recorded, however only at the outer gills, indicating gill damage comes from topical exposure and not orally from hydrogen peroxide. They concluded that the treatment margin around 14°C was very low and that delousing should not be conducted at higher temperatures.

Due to occurrences of high mortality connected to hydrogen peroxide treatments, investigated the connection of algea, hydrogen peroxide concentration, aluminium spores and sea temperatures given at the time of delousing with hydrogen peroxide with salmon mortality. This was achieved by obtaining data regarding these parameters from approxemately 600 delousing operations. The data was somewhat lacking values of the parameters, and all together they were not able to see any direct correlation between any of the parameters and the event of high mortality rates during and after the delousing process. Previous studies however states that use of hydrogen peroxide should not be conducted at water temperatures above 15°C. (Elvin Bugge, 2016) states that they are not conducting delousing with hydrogen peroxide in the event of large quantities of particles present at the location. Measured and determined by the visibility of the surrounding water.

Hydrogen peroxide has also the capability to treat Amoebic gill disease (AGD). The treatment of AGD with hydrogen peroxide requires a much lower effective concentration, around 800 ppm. Which makes a combined delousing and AGD treatment a viable option. However due to AGD the salmon health may be reduced, and treatment at high concentrations of hydrogen peroxide may lead to a high mortality.

Hydrogen peroxide, as an antiseptic, and has the capability of sterilize and enhance salmon welfare when subject to skin damage as of excessive louse levels over a period of time.

4.2 Food supplements

Feed is the most common vehicle for delivery of antibiotics to fish and offers several advantages over topical treatment. The use of feed requires no handling of the fish related to the treatment, which is a huge advantage both cost vise and for the fish welfare. It is also more or less weather independent both operation and environmental vise. However, it requires all fish to consume the food, which renders so called "loosers" which does not feed, untreated. This is also a problem for population which due to a variety of reasons as environmental, stress, etch, does not feed for a period of time. To be effective, the medicine in the feed must be absorbed by the fish, distributed and secreted in mucus for consumption by the target parasite.

4.2.1 Avermectins

The avermectins are a series of drugs used to treat parasitic worms, with potent anthelmintic and insecticidal properties. Anthelmintics or antihelminthics are a group of antiparasitic drugs that expel parasitic worms and other internal parasites from the body by either killing or stunning them without causing significant damage to the host. One anthelmintics derivied from avermectins is abamectin, where emamectin is a derivative og abamectin. (≪Avermectin,≫ 2016) (≪Emamectin,≫ 2016) (≪Anthelmintic,≫ 2016)

Emamectin benzoate is the benzoate salt of emamectin which has been found particular efficient as an insecticide and is the basis of the most used avermectin food supplement under the trade name Slice®. Slice® is meant to be used as supplement in the feed over a duration of 7 consecutive days.

Emamectin benzoate has been shown effective against all stages of mobile stages of L.salmonis and prevents maturation to the reproductive stage. The therapautic margin is high, and during winter has a long effect after treatment. It is reccomended to use emamectin benzoate close to winter/ late fall as the duration of the effect is a function of temperature, as high temperatures result in shorter duration of effect.

4.2.2 Chitin synthesis inhibitors

Chitin synthesis inhibitors has the ability of preventing the formation of chitin, a carbohydrate needed for a insect to form the exoskeleton. With these inhibitors, an insect grows normally until it molts. The inhibitors then prevent the new exoskeleton from forming properly, causing the insect to die. Death may be quick, or take up to several days depending on the insect. Chitin synthesis inhibitors affect insects for longer periods of time. These are also quicker acting but can affect predaceous insects, arthropods and even fish. One of the most common compounds with chitin synthesis inhibitors is diflubenzuron, another well used compound with same chitin synthesis inhibiting capabilites is teflubenzuron.

Diflubenzuron is known under its trade name Lepsidon® and Teflubenzuron as Ektobann®. They are meant to be used as supplement in feed over a duration of 7 consecutive days. Due to their chitin synthesis inhibitors they pose a threat to crustaceans which may be exposed to the supplements. It should not be conducted two feed regimes with chitin synthesis inhibiting feed supplements within 12 weeks as the compounds has a slow destruction rate in the environment, and the accumulation this may lead to.

Chitin synthesis inhibitors is reccomended used when there are few cases of adult L.salmonis present. As chitin synthesis inhibitors only hinders the development from one moult to the next. Therefore closely after the smolt has been stocked into the fish farm, is an ideal time of use as there will be few adult L.salmonis present (Rykhus et al, 2012) 4-5 weeks post stocking of smolt will be a good time for use of chitin synthesis inhibitors, given there is a present copepodite pressure. This is also favourable due to the fact that the total biomass of the smolt is much smaller than slaughter ready salmon and therefore less amounts of Chitin synthesis inhibitors is required. Which leads to reduced amount of chitin synthesis inhibitors used, saving money and environment. The amount of chitin synthesis inhibitors used, is as a function of biomass in the fish cage as the target dose is 10mg per kg salmon (Grant, 2002). It is mostly used in combinations with other delousing agents, after other delousing methods that is effective against all L.salmonis stages has been used.

4.3 Heat treatment

Salmon Salar has as most marine species a temperature tolerance. This temperature tolerance describes at what temperatures it thriwes, and at what temperatures would be lethal. The same is thought to be the case for L.salmonis, however due to its small size and large surface are compared to volume more susceptible to high temperatures than Salmon. The thought is that at high temperatures where the salmon survives, the L.salmonis will die or be paralyzed, loosing its contact with the host.

The method as of today is to expose the infected salmon to water with temperatures of 30°C over a period of 25-30 seconds. The treated salmon and treatment water should be sepparated before re-entering the fish cage as the L.salmonis may be paralyzed and be active after a period of time. (Nygaard et al., 2015) studied the effects on salmon health after exposed to delousing with increased water temperatures, and the efficacy of the method. They concluded that the method had between 75% and 100% efficacy towards the mobile stages of L.salmonis. Several of the test locations had the same levels of adult L.salmonis three weeks post treatment. Which may indicate a lower efficacy towards sessile stages of L.salmonis together with a high copepodite pressure at target locations.

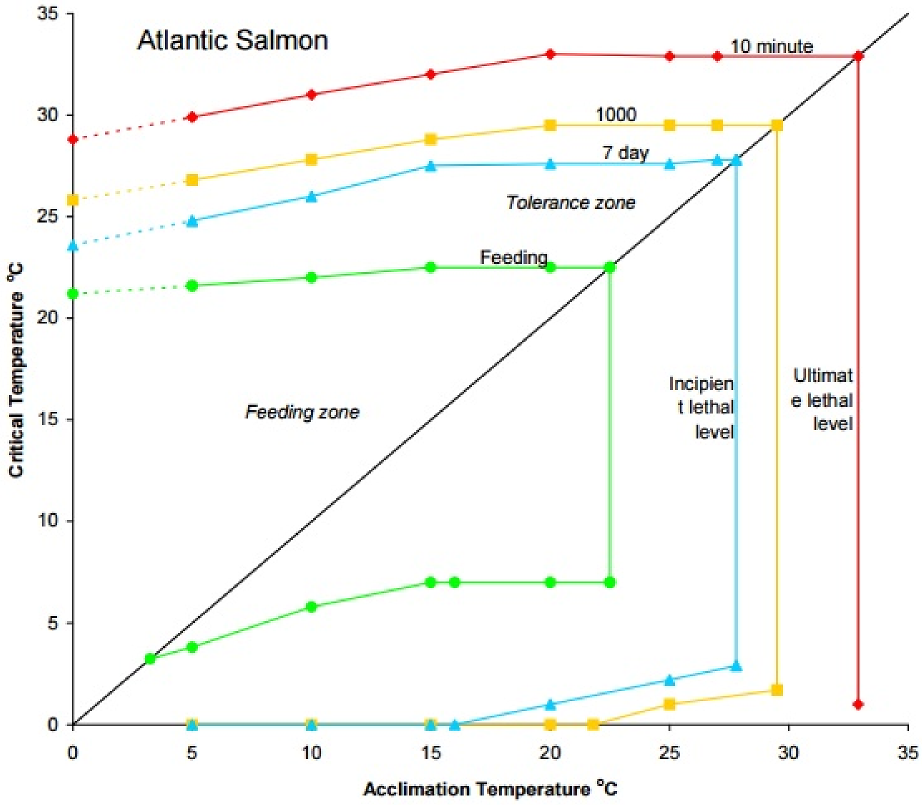


Figure 5: Thermal polygons for Atlantic salmon. (Solomon & Lightfoot, 2008).

Regarding salmon health it is important to see what temperature the thought treated salmon is acclimated to, namely the sea temperature at the location. As it is thought that the sudden change of temperature is of greater importance then the final temperature. Which means greater effect with treatment temperatures of 30°C at acclimated salmon at 10°C than 15°C. (Solomon & Lightfoot, 2008) presented data which shows the correlation between acclimated temperature and corresponding critical temperature for salmon salar. This can be seen in figure 5. This shows that at salmon acclimated at temperatures of 5°C have a lethal temperature of 30°C in contrast to acclimated salmon at 20°C that has a lethal temperature at 33.7°C.

One of the leading products in Norway on heat treatment is the Thermolicer® , whitch pumps the salmon from the fish cage into a pipe system where segments of the piping yielding water with approxemately 30°C before the salmon returns the fish cage.

4.4 Fresh water

L.salmonis only thrive in salt water. They die after a period of time when their adult salmon hosts enter fresh water, depending on their stage in the lifecycle. How long sea lice can survive in fresh water is not clearly determined, as experiments vary alot in results. One study found that most lice fell off and died within two days of being in fresh water (MacVicar, 1997), other studies have shown that over 60% of the lice were still alive after one week and that some survived up to three weeks in fresh water. (Finstad & Bjørn, 1995)

The use of short-term freshwater treatments of Atlantic salmon during the mariculture stage of the production cycle has increased in Norway. Mainly due to the increased occurrence of Amoebic Gill Disease (AGD) and infections with resistant/multiresistant sea-lice. In order to effectively use fresh water as treatment method for both AGD and salmon lice, a number of issues concerning water quality on fish welfare and treatment efficiency needs to be addressed, and knowledge-gaps identified (Powell & Kristensen, 2014) Fresh water is shown to be the most efficient method of treating AGD, with a treatment time of 2 - 3 hours (≪Amoebic gill disease,≫ 2016) and inducing no harm to the fish. However, the handling, grouping, etch, in relation to the treatment does.

Gildeskål (2010) had some experiments where they exposed salmon lice for fresh water, and documented the effect as a function of time. Very clear effects of freshwater were found in reducing both the number of adult male and female lice on infected salmon They showed that exposing the L.salmonis for freshwater over a duration of three hours, reduced the number of mature males by 82.4 % and adult females by 67.7 %. As of these results the adult female is thought as the most resilient towards fresh water of all the L.salmonis stages. The experiments also showed that there were no pre-adult lice on the fish from 60 to 65 minutes onwards in one of the tests, and a reduction from 0.5 to 0.03 pre-adults per fish in the second test.

Fresh water treatment is very similar to bath treatment in methodology, the thought is to have the infected salmon in a closed volume being exposed to a delousing agent for a period of time. However, as fresh water contains no environmental hazardous chemicals, it is often depicted as a mechanical form of treatment due to the denominator of mechanical treatment - no use of chemicals.

4.5 Mechanical

Physical removal methods are bluntly methods which by physical contact removes the salmon louse of the host. All other active methods utilize some sort of reaction which occurs to the L.salmonis.

Few scientific studies has been undertaken on the subject of mechanical delousing, as of what the author is aware of. Perhaps this is due to the crude nature of the techniques, and the mechanisms are well understood. However as resistance towards the methods arise, perhaps a study of the cephalothorax, and the L.salmonis ability to stick onto the salmon should be conducted. Mechanisms reducing the suction capabilities of L.salmonis would be beneficial for all treatmeants, but especially for mechanical methods.

The two most significant physical methods as of today is the use of brushes and pressurized water. SkaMik is a well known technology utilizing brushes to remove L.salmonis. As of 2016 SkaMik has been extensively used along the coast of Trondheim, however reduced efficacy and increased mortality has began to arise in the region, aswell as further north along the coast of Nordland.

SkaMik utilizes brushes to brush off the salmon louse, they report of 90% efficacy at full scale testing. It has the possibility at to treat 270 tonnes per hour and does not depend on temperature. However a healthy salmon pre treatment is favourable as mortality do arise. Little data and research has been conducted on the use of brushes and pressurized water in the manner SkaMik utilizes. Only available data is from their own sites and therefore efficacy and mortality together with period of opperation is not readily grasped. (skal kasnkje stå i diskusjon)

Hydrolicer is another product utilizing pressurized and vacuum to remove L.salmonis. The method utilizes the limiting size of cephalothorax as only this part is holding the L.salmonis to its host. By using vakuum to make the backend of the L.salmonis to be elevated from the salmon, the L.salmonis is more vulnerable to pressurized water. The water is flushed onto the salmon at an angle optimizing the impact area on the L.salmonis and has shown to have an very high efficacy. The damages on the salmon are much less governing than by use of brushes. Little information regarding this product is readily available and information has been obtained through interviews. (diskusjon?)

The potential in developing new methods and optimizing excisting is large on this field of delousing, especially as it is viewed as a ”green” alternativ to other delousing methods as of its no use of chemicals.

5 Passive delousing methods

Passive methods for delousing are basically preventive methods that aims to minimize the amount of sea louse on the fish. Some methods have been used for decades, while some are based on state of the art technological solutions. A brief description of some common methods will now be presented. Defining louse skirt, ultrasound and enclosed fish cages as passive delousing methods may be deceiving as they do not delouse but hinder copepodites in finding a host. However, they are a continous measure to prevent abundance of salmon louse in fish cages and thereby fall into this category.

5.1 Optical Delousing

Using camera vision, advanced software and laser, louse are removed individually from the fish in a gentle and effective manner. Stingray® is the trade name for a known device utilizing laser for delousing. The stingray® contains the camera vision and laser which is lowered into the fish cage. Here it continuously detects attached salmon louse and sends a concentrated laser beam at it. When the laser pulse hits the louse, its blood coagulates. They will not always fall off right away, but they die quickly. Because of the rapidity of the laser, several small pulses can hit each louse while the salmon swim past delousing unit (Stingray Marine Solutions AS, 2016). Little research is known towards the product except their own field tests (Svåsand, Kvamme, Stien, Taranger, & (red.), 2016). They prove that with stingray in the fish farm, the salmon louse abundance is lower over time compared to reference fish cages without stingray. The salmon cages with stingray had a delay of 3 weeks before they exceed the abundance level, compared to the reference cages. The stingray is thought more efficient towards adult and pre-adult L.salmonis due to their size compared to the chalimus stage.

This method is thought of being very gentle in its way of removing the L.salmonis, incidents of eye damage has occured at previous models, but the newer models are guaranteed to be non-eye targeting. They are also beneficial in the way of not being temperature dependant. They will have same efficacy regardless of season and temperature. However, fouling on the optical sensors may be of concern and routine regarding cleaning is needed.

5.2 Cleaner fish

Cleaner fish is a fish that eats L.salmonis of the salmon. Several species of wrasse (goldsinny, corkwing and wrasse) and lumpfish are being used as cleaner fish. Cleaner fish is sometimes in literature appointed as biological delousing tools, and has been utilized to a large extent the recent years(Skiftesvik, Mortensen, & Bjelland, 2015). The cleaner fish has the same function and delousing limitations as the stingray, they both target L.salmonis allready attached to the host.

The cleaner fish is placed in the fish cage together with the salmon and are continuously eating L.salmonis. The continous removal of L.salmonis decreases the overall growth of L.salmonis in the fish cage, increasing the time between each active delousing method has to be utilized. The negative effects of cleaner fish are marginal but cleaner fish nibbling on eyes and fins has been reported. However it is common with high mortality in the stocked cleaner fish (Svåsand et al., 2016). Even though the use of cleaner fish is sought to be a gentle delousing routine, the welfare of the cleaner fish is often discussed, mainly due to the high mortality occurrences. Lumpfish is being more and more utilized as it is active at temperatures below 6°C.

5.3 Ultrasound

Ultra sound is souund waves with frequencies above what human are able of hearing. Ultrasound does not deviate from normal hear-able sound waves in its physical properties, except humans cannot hear it. Ultra sound is normally categorized as sound waves with a frequency of 20 kilohertz and upwards to several gigahertz.

Recently ultrasound has been introduced as a tool against the planktonic stages up to copepodites of salmon louse. A chilean company has developed ultrasound transmitters to this purpose and were rewarded with a price due to the innovation. According to Ltda (2014) they are able to destroy nauplii 1 & 2 and copepodite stages of Caligus rogercressei with ultrasound, the chilean equivalent to L.salmonis. Wether the ultrasound devices has the same effect on L.salmonis is likely but unknown. However, the product is promising with its low cost, no handling of the salmon to delouse and no increased risk of salmon escape.

The utilization of ultrasound is likely to be focused towards the copepodite pressure preventing new L.salmonis to attach in the fish cage. By applying the devices forming a shield around the fish cage will hinder any external pressure in affecting the fish cage. The ultrasound will neither affect the salmon or people working on the farm. Seals and whales will hear them, but prevention of seal coming to the fish cages is only a positive trait.

Ultrasound has been and is being utilized in regard of algae growth prevention. On ship, in pools and other applications. (Mortensen & Skjelvareid, 2015) The mechanism of effect is described to be vibrations which destroy the algae vacuoles, the sound vibrations damages the cell walls, and by cell content grouping in the middle of the cells, preventing the algae in consuming nutrition normally. The vacuoles are small bubbles of air within the algae that provides buoyancy. Aabling and Henze (2010) proved that this effects were easily achieved by moderate ultrasound-intencities, and a single transducer was capable of reducing algeagrowth by 80%. However how compatible these results with algae are to L.salmonis is uncertaint.

A studie undertaken by Guo et al. (2011) proved a clear correlation between use of ultrasound and attachment of barnacles larvae. Surfaces exposed to ultrasound were free of attached barnacle larvae, this however was not due to the ultrasound killing the barnacle larvae, but it was thought to not thrive and settle down where it was exposed to ultrasound. Guo, Lee, Teo, and Khoo (2012) This behaviour is much like the copepodite when choosing host, it can attach to a cod, but after a time of "sencing" its host leave for another host. This however does not validate the use of ultrasound as a shield to prevent the L.salmonis to enter the cage, but rather by creating an unfavourable environment for the L.salmonis to attach and develop in. If this is the case, transducers should be mounted inside the fish cage with applicability to the whole fish cage preventing attached copepodites in remaining at the site.

In the event of utilizing ultrasound to kill already attached and developed L.salmonis it is thought to require high sound pressures, causing cavitation directly affecting the attached L.salmonis (Guo, Lee, Teo, & Khoo, 2013). This is only achievable a short distance from the transducer (10-30 cm), making it near impossible to treat a whole fish cage. It is also possible the cavitation will affect the salmon in a negative manner.

See the report (Mortensen & Skjelvareid, 2015) for a thourough review of the subject.

5.4 Louse skirts

Louse skirts are used to shield the fish from copepodite pressures. By mounting a fine-meshed net around the existing cage structure, the amount of louse registered on the fish is reduced by about 70% (Kvistad, 2016). The effectiveness of this method is varying, and is most likely strongly dependent on factors like local current and wave behaviour (Lien, Sunde, & Bekkevoll, 2015). The louse skirts utilize the copepodite movement which is recorded to be around the surface, therefore the louse skirts are of 8-10 [m] depth, and does not cover the whole exterior of the fish cage. The methodology is that the mesh of the louse skirt is smaller than the size of the copepodite, working as a barrier. However sufficiently large mesh to ensure water circulation and oxygen to the fish inside the cage. Another effect of the skirts is that fouling on the fish cage net is significantly reduced. However, this fouling tend to happen on the louse skirts instead. however it is readily to remove the louse skirt, and replace with clean ones. Which makes the net treatment regarding fouling significantly more cost efficient and easy.

(Næs & Mathisen, 2012) did experiments with and without lice skirts to see the efficacy over a period of seven months. They utilized lice skirts with an opening of 350 µm, which resulted in a decrease of 75% grown L.salmonis after the 7 month period. The fish cages without louse skirts had louse abundance of 0.17 and 0.23 L.salmonis per salmon, the one with had 0.05 and 0.07 L.salmonis per salmon, a significant decrease.

However, the introduction of so fine meshed net pens as the louse skirts are, they often have a solidity around 50% which reduce the throughput of water in the fish cage. This can be especially critical at high water temperatures where the ocean has generally lower oxygen values. The oxygen levels at the different test locations performed by Næs and Mathisen (2012) can be seen below:

	Average (%-saturation)	Standard deviation	Minimum (%. saturation)
Without	111.8	3.6	101.6
With louse skirt	100.6	9.5	75
With louse skirt	102.3	8.5	74.2
With louse skirt	104.2	10.3	67.1

Table 3: Oxygen saturation with and without louse skirt (Næs & Mathisen, 2012)

Growth vice the experiments could not conclude that applying louse skirts would result in lower growth than those without. On the contrary the one with greatest growth was one with louse skirt, but also the one with lowest growth had louse skirt. The one with lowest growth rate did however not deviate mentionable from the one without, as these had larger fish stocked at the beginning of the experiment compared to those with greatest growth. Mortality there very no noticable difference between the fish cages with and without louse skirts.

5.5 Alternative cages

As of today, the most common fish cage utilized in Norwegian mariculture is the open circular fish cage utilizing elastic plastic floaters and net pens, so called floating net pens, or net pens. Net pens are considered a high impact aquaculture method because the generated fish waste passes freely into the surrounding environment. There is also a considerable possibility for the stocked fish to escape. Diseases and parasites also spread in and out of the net pens. These arguments are heavily discussed and an ongoing debate between the pro-wild salmon and the fish farm companies. The spread of parasites is already being covered and the open nature of the net pens undoubtedly enables the spread of parasites as L.salmonis. This and the previous effects of net pens has lied the foundation of closed sea cages. These closed cages are cages that swap the enclosing net pens with solid matter, which enables regulation of all aforementioned aspects. There is an ongoing technological revolution as of alternative fish cages going on in Norway. As mentioned no new regular licenses will be stated as of today, however so called "green licenses" are being stated. In order to be granted green licenses, the concept must cope with the governing environmental aspects sufficiently. Therefore several concepts of closed, exposed and submersible fish cages are being developed and some granted licenses. All in order to increase produced biomass, without increasing the negative environmental impacts caused by the production. However, there are several implications with these new fish cages. Implications which often is highly costly economically, both operational and construction vice. The high salmon price and large costs related to parasites has however been estimated to cover these expenses, which has lead to this boom of new concepts. All three categories has the common idea of being louse free, however the submerged and exposed cages may not have this as a governing parameter compared to the closed cage concepts.

5.5.1 Closed cages

Closed fish cages are fish cages where the net pens has been replaced by a solid material, generating a totally enclosed volume. The in and out flow of water is handled differently from the different concepts, however hindering influx of copepodites while satisfying oxygen recirculation is the common denominator for all the concepts. Pumping inlet water from depths of 20-30 meters will reduce, but not necessarily eliminate content of louse in the water. By treatment of outlet water, spread of other fish diseases can be prevented (Teknologiraadet, 2012). The fish waste is also being collected, enabling the closed fish cages to be located close to the shore or other beneficial locations which else would be affected by the large accumulation of fish waste.

Drawbacks with this technology is an increased complexity of the systems, larger loads from the environment, requirement of new operational routines and high production costs. There have also been documented issues regarding fish welfare in previous test projects on closed fish farming (Teknologiraadet, 2012). The handling, grouping, and other procedures previously accomplished by penetrating the net pens, has induced some problems and are solved differently from the different concepts. The main difference between the closed cage concepts is the planned operation of the fish cage related to fish handling, waste removal and inlet/outlet for water circulation. Otherwise, the idea behind the concepts is the same, control in and outlet water and fish waste removal.

5.5.2 Submerged cages

As stated in the copepodite section, the copepodites are usually located within a 10 [m] depth relative to the surface. By lowering the fish cages 10 or more meters below the surface, it is believed that the copepodites will float ower the cages, reducing the amount attaching to the fish within the cage. This concept is also proposed to exposed facilities as the wave forces decrease exponentially with water depth, which is the main factor of the net pens being located at sheltered locations as of today. There are several implications with submerging the fish cages. First of all the salmon has a swim bladder which it needs to regulate, requiring access to the surface. The salmon is also light dependant, altering growth and eating habits. The fish handling is also a large concern, upon being handled the fish cages must be raised to the surface. The concepts planning submerged solutions have often implemented artificial lights and pockets of air to cope with these problems.

5.5.3 Exposed mariculture

The general idea of exposed mariculture is to utilize locations previously unfavourable for mariculture, and by this also avoid being exposed to copepodite pressures. Avoided simply by its location far from any other fish famrs which may have L.salmonis infection. Salmar a large salmon producing company in Norway has gotten approval of its large project regarding open ocean cage farming. Where "one" cage contains licenses to hold up to 8000 tonnes. This concept will be discussed in the discussion section regarding its planned location, and the present copepodite pressure.

6 Results

In order to solve the problem regarding the L.salmonis, one has to understand the L.salmonis. By understanding its mechanisms one are able to create methods of removing it. In the biologi part the biologic aspect of the L.salmonis with its different stages and capabilities were described and the most important result is how it spreads. By understanding that the spread of L.salmonis is not within a fish cage but from an externally generated copepodite pressure, one will understand the importance of a regional delousing regime. As well as the benefits from passive methods that hinder the copepodite pressure in attaching in the fish cages.

The rise of resistance towards some of the active delousing methods also implyes a need for regulations towards massive use of a single active delousing method at the time. The different methods does allso have different capabilites at certain aspects, some are rendered useless at some water temperatures, while other are functional all year around. This knowledge should be utilized in order to achieve a optimized strategi towards the salmon louse. A rotational system and logging of what measure has been utilized where can be and will be beneficial in preventing further increase of resistance, and achieving a much higher efficacy of active methods on average.

As the salmon louse spreads through water currents in its early stages, it has been possible to generate a simulation combining ocean currents along the Norwegian coast with reported salmon louse abundance at the fish farms. Ø. Karlsen (2016) has generated a simulation model which visualize and generates the expected copepodite abundance in the water at any location along the Norwegian coast. Havforskningsinstituttet generated this model on basis of reported louse abundance to Mattilsynet and the ocean current model called NorKyst800. NorKyst800 is a model developed by Havforskningsinstituttet in cooperation with Meteorologisk institutt and NIVA. The salmon louse pressure model presents the estimates on a visual map over the Norwegian coast with colours indicating the estimated copepodite abundance per square meter at a given time. The model has a preciseness of 800 square meter, meaning the coast is divided up into squares of 800 m^2, and abundance estimated per square meter.

As this model gives an estimated copepodite pressure on all locations along the coast where there are located fish farms, one has a good indicator of what is to come and should utilize this knowledge to start preventive measures and/or plan other measures. This can be done by simulating the development process of L.salmonis as a function of time, sea temperature and copepodite pressure. A simmulation model can be very handy to estimate future L.salmonis abundance to give the operator a heads up regarding the need for an active delousing method in the future, before the abundance has violated the limit, and there may be no available vessels to delouse.

6.1 Simulation model

Based on gained knowledge a simulation model has been created which simulates the direct consequence of the copepodite pressure and what tools that is at hand for a whole region. The problem can roughly be put as: there is a copepodite pressure affecting our fish farms, how do we cope?

The simulation system can be divided into 4 segments:

- The copepodite pressure at the given fish farm site, and its growth into mature reproductive L.salmonis
- The active delousing methods which are available in the region at the specified time.
- The preventive measures which is possible to utilize
- The salmon with its health and growth aspect at each farm.

These systems are well investigated, it is however their interaction with each other which are uncertain and of great interest. These interactions can be treated as stochastic variables as we do have some data regarding some of the aspects, but nothing certain.

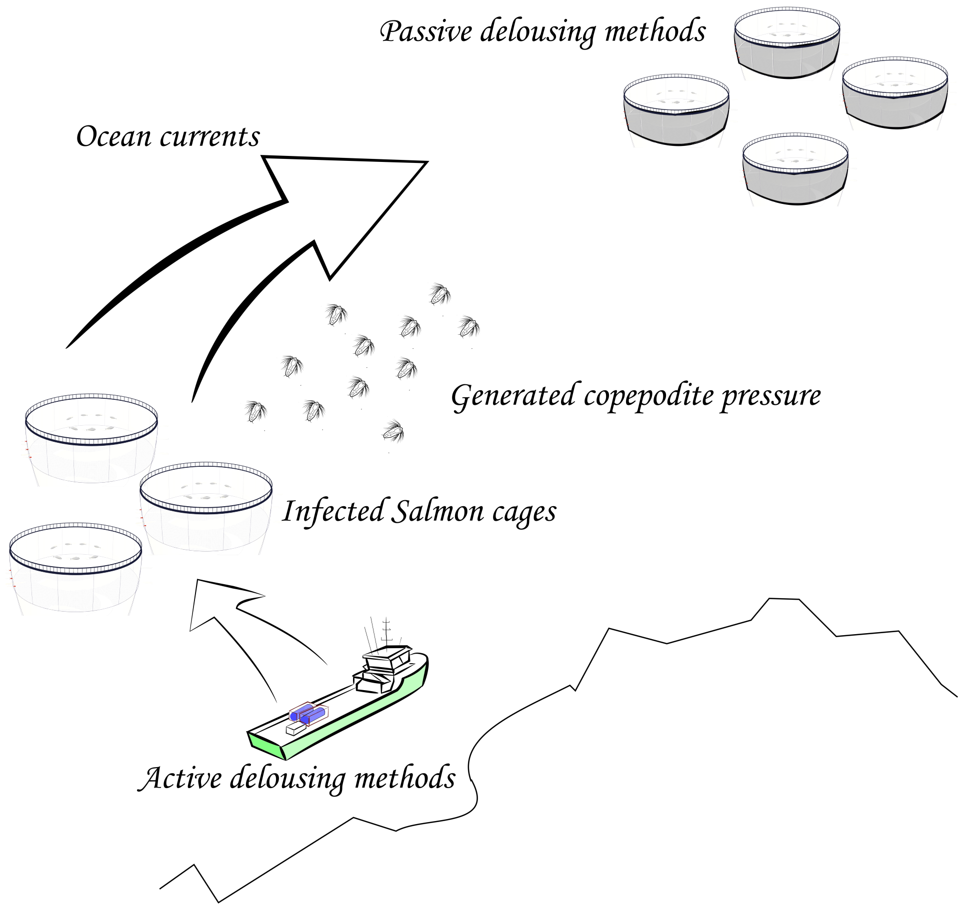


Figure 6: Illustration of the simulated system

What we want this model to give us, is an indication of what measures should be taken in case of an copepodite pressure, and when. This is found by estimating the optimum tolerance limit for a salmon cage regarding abundance, at what abundance should an active delousing method be utilized. Is the introduction of passive methods cost efficient as of their efficacy and spread to other farms. The idea behind the system simulated can be seen in figure 6

6.1.1 Salmon subsystem

The salmon present at a fish farm is modelled as entities which yield a set of characteristics. The initial number of entities are how many smolt which are stocked at the fish cage, at that specific time. Its initial characteristics is mainly how big the smolt was at the stocking. The salmon then grows as a function of time and sea temperature. It is assumed that the same feed is fed to all the cages resulting in the same feed related growth, this however can readily be modified. In the event of an increased L.salmonis abundance the growth decreases according to recorded growth decrease related to L.salmonis attached. In the event of active delousing method being utilized the fish is not fed and does not grow in a period of time prior to the treatment. These starvation periods are chosen from current practice regarding each active delousing method. Upon being treated there is linked a certain random mortality, the magnitude of mortality and the nature of the stochastic variables differ from method to method and are often correlated to starvation period and especially sea temperature.

This model gives an estimated time for the mariculture segment in the salmons life cycle, and how much biomass is estimated to be present at the fish cage at any given time. This sub-model alone can prove valuable for production managers in estimating time of stocking, when to slaughter the cage and what status quo on biomass is at any given time. The model is readily to modify adding more variables regarding the salmon growth or other aspects affecting total biomass at the fish site/cage.

6.1.2 Copepodite pressure and L.salmonis development

The perhaps most powerfull tool in the simulation is the utilization of the estimated copepodite pressure at any given fish farm. As mentioned the L.salmonis only arise at fish farms from attachment of copepodites, therefore noting a high pressure at a site, gives a strong indication of that a L.salmonis abbundance is imminent. The estimated copepodite pressure is taken from Ø. Karlsen (2016) which change as a function of time and the abundance of the fish farm/farms of its origin. The copeopodites are then modelled as entities yielding characteristics inherited from the L.salmonis abbundance which generated it. If its origin is from an abundance whom post a completed active delousing method, the characteristics is then increased tolerance to that specific delousing method/methods. These methods therefore will not have the same efficacy as to L.salmonis without these characteristics. The copepodites attached in a fish farm develops through its lifecycle as a function of time and temperature according to table 1. Upon being treated with an active or passive delousing method each L.salmonis depending on its stage, characteristics and what delousing method being utilized renders the possibility of dying. The mortality of L.salmonis has a stochastic variable attached to it, as the efficacy of the delousing method is never 100%. Upon surviving a delousing method without having any characteristics related to the method utilized, this new characteristic is "discovered" and implemented to the L.salmonis. This has the possibility of creating multi resistant L.salmonis which neither of the methods has any high efficacy against. The probability is however low, but upon maturation and reproducing these characteristics will spread through the generated copepodite pressure to other farms further downstream. The L.salmonis can however not "discover" tolerance towards cleaner fish nor stingray, as this has not been observed and is deemed a bit strained. The idea of spread characteristics through reproduction can be seen in figure 7

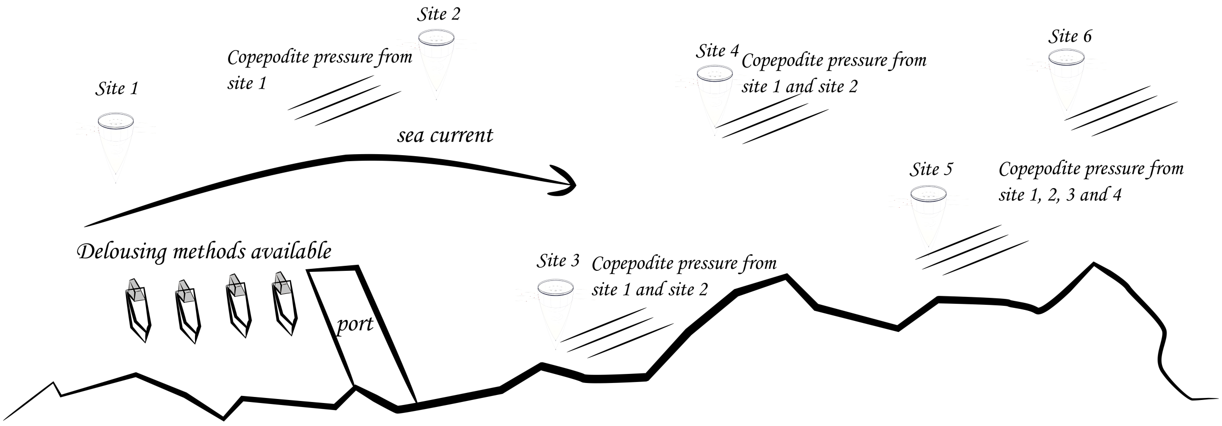


Figure 7: Copepodite pressure generated by several sites

6.1.3 Active delousing methods

The active delousing methods are called upon by the fish farms. This can be done when the fish farm has abundances above a set limit, or as a preventive measure. Each delousing method represent a ship and has the characteristics of what delousing method it has implemented. The ships characteristics are typically rate of delousing, meaning how many tonnes the method can delouse per hour, determining the estimated stay and occupancy at a site. Other characteristics is also the vessels estimated speed, which typically varies from the barge formed ships utilizing brushes to the modified PSV's carrying hydrogen peroxide tanks. The delousing methods can readily be modified in numbers and characteristics as new vessels are introduced into the market.

6.1.4 Passive delousing methods

Passive delousing methods are applied at each specific fish farm site. These include the introduction of stingray, cleaner fish and louse skirts. The Stingray and cleaner fish are modelled allmost equally by continously removing grown L.salmonis in its designated fish cage. The cleaner fish's efficacy is however a function of sea temperature as it has been recorded lower efficacy at low sea temperatures than otherwise. The stingray has the same efficacy regardless of sea temperature, but will not have any efficacy towards L.salmonis in stages pre adult stage. The implementation of louse skirts at each site reduces the attachment at the site from the adjacent copepodite pressure. This has been modelled through hindering a given number of copepodites and in the event of a copepodite pressure exceeding this number, it hinders 60% of the copepodite over this number in attaching inside the fish cage. For example a copepodite pressure of 17 copepodites per m^2, the louse skirt hinders 7 + 10*0.7 = 14 L.salmonis in attaching within the cage. Leaving 3 to have the chance to develop.

6.1.5 Simulation results

It is favourable to run the simulation over a period of some stocking cycles, typically 5-6 years. This is in order to see the effect of fallowing the farm sites after ended stock cycle. One stock cycle typically lasts for 1.5 years however prone to variance due to variations in stocking size, water temperatures etch. The simulation results does not show what the optimum number or type of delousing to perform. It simply estimates the result of set parameters prone to stochastic variables. We are however able to alter these variables and compare the simulation results of the different settings. This enables further optimization towards the best variable settings at the given conditions for the region. The simulation results we are interested in regarding each of the fish farms and the individual delousing vessel, these are:

- L.salmonis abundance, adult and pre-adult stages.
- Number of active delousing performed, when which method was used, how many times over the simulation duration each method has been utilized and corresponding delousing efficacy.
- Number of stocked salmon, duration from stocking to slaughter, and resulting slaughtered biomass

These results are intertwined and dependant on each other, many delousing procedures usually result in loss of biomass but also reduced L.salmonis abundance and so on. The interconnection and variable path can be seen illustrated in figure 8 The simulation results has been plotted and can be seen in appendix C.

Based on the origin of the copepodite pressure one can readily see what method not to be used on the different farm. A copepodite pressure arisen from survivors of one method renders the efficacy of the same method poor for the developed L.salmonis. Implying the need for a rotational system utilizing different methods which affect different mechanisms of the L.salmonis. The main results from the simulation is the abundance of adult L.salmonis at each farm site as a function of time, together with what and how many times the active delousing methods has been used. Altering the set variables is readily and can result in interesting results. By altering the abundance limit of 0.5 salmon louse per salmon one can see whether the set limit is optimum or not.

The simulation model with its result form a powerful and readily tool for a person responsible for the fish welfare in a production company. They can identify what methods the L.salmonis responsible for the copepodite pressure at any given fish farm site has been treated with. Their delousing strategy can be tested through the simulation, enabling several plans to be tested and the results used as indicators of what measures to be conducted. The model will be in real time, enabling ongoing simulation on basis of taken measures. However, for the simulation model to be used as intentioned, alot of work has to be conducted in order to form valid variables used.

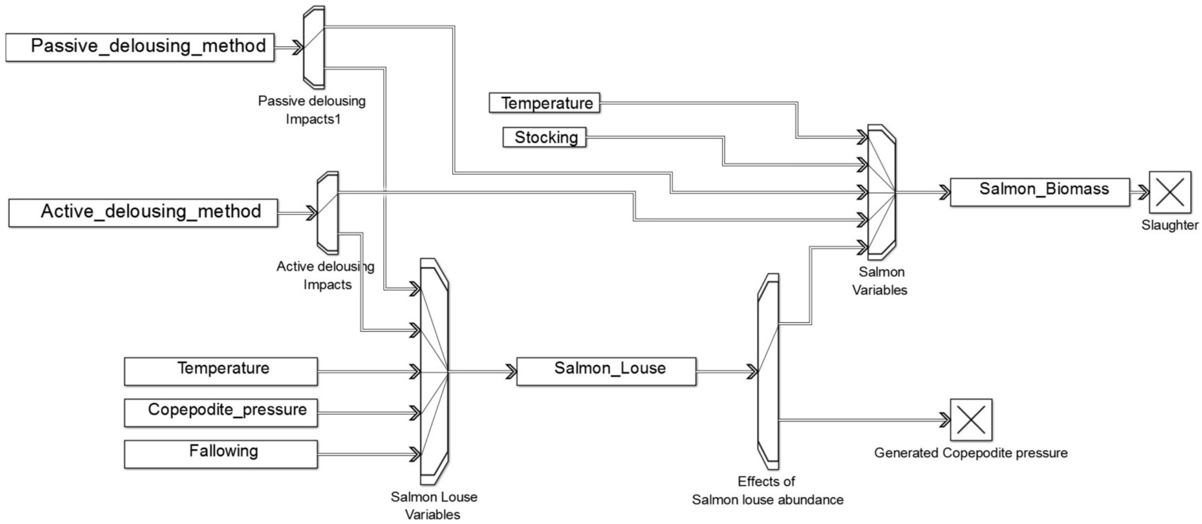


Figure 8: Illustration of interconnection of the most governing stated simulation variables

6.1.6 Stochastic variables

There is little to no reason to simulate something if there are no stochastic variables affecting the output. In the simulation model there are plenty. It is important to have a grasp of what these variables are and how they are described mathematically. Regarding the copepodite pressure, these numbers are just collected from (Ø. Karlsen, 2016) model for each site, varying with time according to their estimates. The very nature of these numbers are complex and based on uncertain variables. As I did not develop this model, I will not go into further detail, but the basis of the model is thoroughly explained in their own report.

Both Salmon biomass and L.salmonis are strongly temperature dependant, therefore as real as possible values of the sea temperatures is important. This is achieved by forecasting the sea temperatures at each site/area on basis of monthly recordings back to 1946 by use of winter's forecasting model. This model takes seasonality, trend, and levelse into account and deemed as the best forecasting method for my use. There is some uncertainty regarding the impact of the temperatures, therefore the variables related has been added a random variable determining the the size of the standard deviation to be accounted into the thought mean impact. The development of copepodite characteristics is purely random where each Copepodite has a small probability of having one or more of the delousing resistance characteristics. This is thought to be removed as function of time, or if we are to find status quo of the methods used at the origin of the different pressures. Their development and chance of "discovering" their resistance is also a random process. The effects of delousing for each method has been weighted somewhat different between the different methods, and with a certain random variation to their efficacy.

This can be improved by gathering real data. Operation vice, the weather is of great importance, however implementing realtime forecast has not been implemented but replaced by a random seasonal variation. This is thought to be readily to implement compared to some of the other variables. A graphical illustration of the different variables and their respective impacts can be seen in figure8

6.1.7 Optimization algorithm and result analysis

As the model previously explained only simulates a certain outcome of a given situation based on realtime inputs, it is not readily to know what measures are optimum for the different fish farm sites. Therefore the simulation should be incorporated in a non-linear optimization method. Due to the non-linear nature of the problem this optimization method use brute force by simulating a large number of simulations and optimizing the parameters resulting in the best simulation outcome. The choice of non-linear optimization method can be as crude as sorting the results from thousands of simulation and pick the one resulting in lowest costs, but due to the massive simulation model this requires an enormous amount of equations to be solved. And it is therefore recommended to use a more clever non-linear optimization method as for example a particle swarm optimization method.

6.2 Rotational system

A L.salmonis surviving a delousing method, has good possibilities of surviving the same method again in its life span. Given that the reason for survival the first time was not error in delousing method handling, rather an increased characteristic beneficial towards the method compared to other of its kin. These characteristics are not necessarily developed as a direct result of the delousing method, rather inherited characteristics. Given that one male and female both survive a given method, upon copulation and spread, their offspring is made of their characteristics and will therefore most likely have good survivability towards the same delousing method (Darwin, 1859). The generated copepodite pressure will therefore have characteristics of increased survivability towards the specified delousing method. The fish farm sites exposed to the pressure will therefore experience low efficacy of delousing with same method utilized at the copepodite pressures origin. This has been observed along Nordland with Mechanical delousing efficacy down to 30%, perhaps as a result of the extensive use of mechanical delousing along the coast of Trondheim. Plausible as the ocean currents are known to go along the coast from south towards north. And is the spreading mechanism of the L-salmonis.

Extensive use of same method at same fish farm site and region poses therefore a threat to itself and the region exposed to the generated copepodite pressure. This Implies the need of altering methods from one delousing to the next at the location, preferrably a different method compared to the present L.salmonis origin. The new method should be different from the previous method as in mechanism of removal. Changing from mechanical brushes, to pressurized water has little purpose as the L.salmonis characteristic favourable towards brushes is also favourable to prerssurized water, its clasping capability to the host. Therefore changing to a chemical, thermal or freshwater method is a preferred choice. This way of thought enables a rotational regime, where the utilization of the different methods is not only governed by their availability or constraints, but also the fish site/regions delousing history. In the event of fallowing, the history of delousing methods can be erased. Assuming all L.salmonis with previous characteristic is gone from the site. The copepodite pressure with its given characteristics however may not be assumed removed, unless its origin also has undergone a period of fallowing.

The simulation result is heavily influenced by the concept of "discovered" characteristics, and will automatically utilize different delousing methods within their constraints. This can however readily be overwritten to show the thought affect of "dumb" delousing strategies. Dumb as in the sence of random choice of delousing method regardless of constraints or delousing history.

6.3 Utilization table

As stated previously, the different methods has some variations in when to be utilized. In table 4 and 5 the different methods has been characterized whether it should be used, or not, depending on given month. The month dependency is on sea temperatures, and it should be taken into account that temperature variates not only with month but also location. The tables therefore should only indicate the methods restrictions and applicability regarding its temperature sensitivity. Yes means that the method is applicable in during this month, no means it should not be utilized, care indicates that it is not favourable to use this method, and extra care should be taken if the method is used.

Table 4: Suggested use of delousing methods, January to june

	January	February	March	April	May	June
Brushes, pressured water	Difficult due to weather		yes	yes	yes	yes
Fresh water			yes	yes	yes	yes
Heat treatment			yes	yes	no	no
H202			yes	yes	care	no
Pyretroids			care	care	yes	yes
Azametiphos			yes	care	care	no
Avermectins	yes	yes	yes	yes	care	no
Chitin synthesis inhibitors	yes	yes	yes	yes	yes	yes
Cleaner fish	care	care	yes	yes	yes	yes

Table 5: Suggested use of delousing methods, July to December

	July	August	September	October	November	December
Brushes, pressured water	yes	yes	yes	yes	yes	yes
Fresh water	yes	yes	yes	yes	yes	care
Heat treatment	no	no	care	yes	yes	yes
H202	no	no	care	yes	yes	yes
Pyretroids	yes	yes	care	care	no	no
Azametiphos	no	no	no	care	yes	yes
Avermectins	no	no	care	care	yes	yes
Chitin synthesis inhibitors	yes	yes	yes	yes	yes	yes
Cleaner fish	yes	yes	yes	yes	yes	care

7 Discussion

There has been a strong focus on achieving objective information regarding the biology of the salmon louse, and the description of the different delousing measures. Everything stated about these matters has been constructed through my interpretation of others work, and misinterpretation of information may have occurred. Especially regarding the biology aspect, due to the advanced nature of the papers/articles investigated. Information stated about a delousing measure by the company behind it has been deliberately avoided as their stated efficacy and applicability often deviate from scientific papers. For the case of Skamik/brushes in general, it was hard to find any research papers on the matter, and therefore the stated efficacy and applicability should be viewed thereafter.

The constructed utilization table has only focused on the water temperature dependence of the different delousing methods. There are many other variables which come to part regarding choosing one method over the other which has not been accounted for here. Therefore in the event of choosing a delousing method one should view the table as an indicator of which methods to consider, and not be the basis of decision.

The simulation model created is fully functional, and has the capability to readily implement advanced variables regarding the many aspects of the model. The variables already implemented however lack the realism desired to simulate good estimates. The model should therefore be viewed as a platform for further work where research conducted on the different variables can gradually be implemented as they are developed.

The overall complexity of the description regarding the delousing methods mechanism could be further improved. As the mechanism of why they are effective against salmon louse is important to understand. These mechanisms were however deemed on a general basis as too complex and tedious to describe due to the many complex biological terms and conflicting scientific reports.

7.1 Salmon production

In the salmon production section it is stated that it is only possible to stock smolt from hatcheries for a short period of time two times a year. And the reason was due to the smoltification process. However, the smoltification process has been recorded to be prone to manipulation, enabling the smoltification to reverse and smolt again several times over a period of time. This would imply that there are possibilities for stocking in addition to the stated period of mai and september.

There may also be some misunderstanding between the production of salmon and the production process. Production of salmon is related to the shear biomass production phase of the production process, where the production process is the whole cycle from brood salmon, egg to slaughter. Salmon production companies are companies which either control the whole production process cycle, but most commonly known as companies whom hold licenses and are producing salmon in the mariculture phase.

7.2 Biology of the Lepeophteirus salmonis

It should be noted that the author is studying for a master of science within marine technology. My specialication is within marine resources and mariculture and several courses governing salmon biology and production has been taken. Therefore the content of the biology part is not necessarily too interesting for a biologist with in depth knowledge of the L.salmonis. However, the L.salmonis mechanisms of particular interest has been investigated thoroughly and present several aspects which is deemed interesting related to the actual L.salmonis Salmo Salar interaction. Some of the particular mechanisms may have been misinterpreted wrong, but alot of work was done to avoid any misunderstanding between literature and my representation.

7.3 Simulation model

The simulation model is built up of several components all prone to discussion. The overall quality of the model relies on the validity of each component, and one can say that the overall error of the model is the accumulated error from every component. It is therefore important to investigate and expose flaws in each component which contribute to the overall quality. By identifying these flaws it is readily to plan further improvement of the components and therefore the model. Due to the uncertainty of the many components within the simulation model, the simulated results should not be used in any decision making related to delousing. The simulation results can however simulate the effects of altering variables, and see the thought effects of different variations. in the event of more realistic representation of the variables, the simulation model can very much be used in planning of delousing. Which is the goal for the simulation model.

7.3.1 Copepodite pressure

The copepodite pressure is modeled through simulation results gained from Ø. Karlsen (2016). The people behind the estimate do claim that improvements are needed as some other models has had better estimation accuracy. (Ø. Karlsen, 2016) has done a thourugh job in creating a simulation model based on Ocean current simulations and the abundances of L.salmonis at the fish farms along the Norwegian coast. In my model these estimates has been collected at the farms investigated and implemented into the respective farms in my model. These numbers are not updated continously through the estimates from Ø. Karlsen (2016). And therefore is not deemed as accurate for simulation further in time than the given time when the estimates has been collected from. A clear improvement would be to obtain the simulation model they have produced, and implemented into my model with some adjustments which will be discussed.

The development of characteristics through the generated copepodtie pressure is not included in the model developed by Ø. Karlsen (2016). My implementation of this pressure disregards all aspects and is an crude function of a constant sea current from one fish farm site to the next. What would be interesting was if I were to obtain (Ø. Karlsen, 2016) simulation model and implemented the spread of characteristics and implement this model in my model. Then one could clearly identify fish farm sites which are affecting the largest number of fish farm sites, and experiment by reducing tolerance levels or increased measure on these locations and seen what effects these had.

7.3.2 Stochastic variables

As stated the simulation model relies on the varibles describing the different subsystems in order to create a good estimate. The most important variables in the model is deemed to be:

- Temperature
- Weather
- Growth: L.salmonis, Salmo Salar
- Operation time for each delousing method
- Vessel movement
- Delousing methods efficacy
- Salmon death related to delousing

There are several existing models forecasting sea water temperatures and weather. This should be readily to implement correctly into the model. However computational vise it must be done in a smart way to prevent unnecessary amounts of computations related to the implementation. This could be done by generating a script which prior to the simulation has estimated these variables. This script would continuously update the estimated values, to prevent simulating on water temperatures for October based on data from February, when there are available estimates from September.

The growth and development of salmon louse and salmon salar is strongly temperature dependant. There has been conducted a lot of work related to this correlation, and it should be readily to create accurate variables in the simulation model. The correlation was found to be described differently in several scientific papers, and it was not readily to create an immediate correlation in the model. Further work on this subject should result in an pretty accurate variables.

The availability of active delousing methods is limited, which is often a problem during the high season of salmon louse abundances during fall. In order to simulate this availability accurately it is crucial to figure out the estimated time of operation for each method. As these methods are utilized as of today, this implies that there exist real numbers on the matter. Therefore collecting data regarding operation time and estimated time until a new operation can be underdone should be possible to obtain.

The previous variables has been deemed relatively readily to obtain, just dependant on a lot of effort put into the data handling and representation in the model. The variables regarding delousing efficacy and salmon death related to delousing measures however, is connected to a high degree of uncertainty. The delousing efficacy of each method relies on the characteristics of the salmon louse. The characteristics favourable towards some of the delousing methods has not yet been identified. An estimation of inherited characteristics has formed the estimation of the salmon louse sensitivity towards the different methods. If a fish farm site has 30% efficacy with a method, the salmon farm which is affected by its copepodite pressure will then experience the same efficacy with the same method. There has also been recorded large quantities of salmon death in relation to delousing procedures. One method can in one instance have high efficacy with marginal loss, and in the next instance kill 90% of the treated salmon. Identifying the factors connected to these high mortality rates is of high interest for the industry. Some of these variables has already been identified, but there are still reports of high mortality related to delousing.

7.3.3 Computational efficacy

As the simulation model is quite complex, the number of equations to be solved is even more comprehensive. Each salmon louse per fish farm has been modeled, and each of these entities are prone to multiple alternations over to course of the simulation. When one start adding more farms to the simulation model, one run may take many minutes to solve. This is not optimal as the optimization would require a lot of simulations to have any validity. For further work the shear logic behind the modelling of the salmon louse should be more cleverly executed, to save time and to ease the workload in relation to trouble shooting and overall simulation efficacy. Cleaver improvements as in reducing the number of equations to solve without decreasing the accuracy of the simulation results.

7.4 Phraseology

In relation to this thesis the words salmon louse, Lepeptheirus salmonis, L.salmonis, salmon lice, delousing method, delousing measures, delousing, active and passive delousing methods. Has been used in an un-orderly fashion at times. Especially confusing may the mixing of delousing methods and measures be for those without prior knowledge to the subject. Delousing measures may be viewed as all measures which combat the salmon louse, from preventive, active, passive methods etch. Delousing methods has been used in stead of delousing measures in order to exclude preventive measures such as land and closed cage based production. The differentiation is deliberate as it is deemed un-logical to implement land based production in a mariculture.

8 Conclusion

A thorough overview of the current delousing measures has been stated, the different measures has been categorized based on their usage, applicability and constraints. The biology of the Lepeophteirus salmonis has been investigated and described in relation to the problem it poses. A simulation model has been created aiming to estimate the consequences of salmon louse abundance on a regional level. The simulation model does not develop any valid estimates as of current state, but has great potential in the event of more realistic variables being implemented. The thesis is deemed to form a solid foundation for further work in relation to the salmon louse problem.

9 Further work

As stated in the discussion, the thesis and especially the simulation model has a large potential for improvement. The potential varies from the biology of the salmon louse to construction of the simulation model. The scope of further work may be deemed as to large for a single student to comprehend in a master thesis, and may require additional master thesis's in order to cover the scope sufficiently.

9.1 Salmon louse biology

The salmon louse has been investigated and what deemed as most important related to the thesis stated. There are however several aspects which has not been investigated as thoroughly as desired. The mechanism of how the copepodite attaches to its host, and how it gains a permanent attachment has been shallowly described. There are papers explaining this mechanism in depth which was not stated in this thesis due to its complex description. In further work this should be investigated further and stated in an understandable manner. Another aspect needed to be investigated further is how the copepodite finds its host. In the thesis it has been described though sensing shack waves generated by the salmon. I have understood that this is not commonly accepted as of how it finds its host, as many papers state this as an uncertainty. Work related to determining whether this statement is valid or not should be done.

9.2 Delousing measures

There are several aspects of the delousing measures which has not been investigated sufficiently in this thesis which is deemed important for the overall goal. Each measure should be investigated related to:

- Cost of implementation/execution
- Availability
- Operation/installment duration
- Historic delousing efficacy
- Salmon related mortality

9.3 Simulation model

The simulation model is prone to alot of improvements. The most important aspect is the development of more realistic variables as described in the discussion. Further improvements to the model is implementation of characterisics in the copepodite pressures, enabling real-time input, refining the stochastic distributions and presenting the simulation results in a soothing manner for commercialization. The coding behind the simulation could also be further improvement, reducing the number of equations to be solved without compromising the simulation results.

9.3.1 Estimated copepodite pressure

As of now the desired copepodite pressure estimated on basis of (Ø. Karlsen, 2016) including inherited characteristics does not exist, as of what I am aware of. What would be desirable is to access the simulation programme developed and look into the possibility of implementing characteristics in their existing model. This type of copepodite pressure estimates would be of importance as of the functionality for my simulation model. However disregarding characteristic based pressures may still form imprtant results, as one still has the estimated abundance of L.salmonis as a function of all the named parameters. This has to some extent already been implemented in the model.

9.3.2 Real time input

The three variables which already has been estimated by external models is the copepodite pressures, sea temperatures and weather. These estimates are estimated on a daily basis which would be convenient to be implemented in the model. By implementing new estimates on a daily basis will reduce the induced error from these variables as the estimates shift on a daily basis. In the event of an delousing measure being used at a fish farm in the real world, would affect the fish farm and should be implemented in the model. Typical alterations due to these measures are new salmon louse abundance, at the specific fish farm.

9.3.3 Mechanical resistance

To now it is has been a common thought that resistance towards mechanical delousing methods as brushes and pressurized water is unlikely. However, recent events has proved otherwise as discussed. This resistance should be readily to measure as the resistance is linked to what force they are able to withstand before being removed. Creating a louse weight, functioning as a tweezer, measuring the force required to remove the L.salmonis from its host. Would be of great use to see the variation in suction capability along the coast. Determining the impact of the massive mechanical delousing regime ongoing along the coast of mid-Norway. Which should be implemented in the model as of the efficacy towards mechanical delousing.

9.3.4 Graphical interpretation

For the common man the simulation model and optimization result may be deemed as cluttered and not user-friendly. However by creating a interactive map over the coast showing the properties of the fish farm sites, with environmental properties, L.salmonis abundance etch. Together with shifting weather and the estimated copepodite pressure with its varying characteristics. One would be able to show the potential and applicability of the simulation/optimization model properly.

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NTNU
Norwegian University of Science and Technology
Department of Marine Technology

PROJECT THESIS

A Alternative thesis front page

Title:	**Delivered:**
Simulation-based decision support system for delousing of Lepeophteirus salmonis	16.12.2016
	Availability: erikanae@stud.ntnu.no
Student: Erik Andreas Næstvold	**Number of pages:** 45

Abstract:
Norway's aquaculture industry has through the last decade experienced an explosive growth. Total produced salmon biomass for 2015 had doubled compared to 2007 and the net export value tripled. The industry seeks to grow even further, and by 2050 it is sought to produce five times the biomass compared to 2015. This goal is highly dependant on what measures have been developed to reduce the environmental impacts of aquaculture, as no new regular production licenses are stated from the government until the problem has been sufficiently dealt with. This environmental problem is mainly related to the salmon louse, lepeptheirus salmonis, and its impact on wild and stocked salmonids.

Lepeptheirus salmonis lives as an ectoparasite on salmonids, where it feeds on mucus, tissue and occasionally blood. The impacts on the salmonids is tissue damage which increases its susceptibility for secondary infections, reduces overall health, and induce stress. Which in addition to other health aspects may lead to death. The vast numbers of salmon along the Norwegian coast has made the spread of salmon louse readily due to the large number of potential hosts. There has been developed several methods to combat the salmon louse, and the salmon louse has been estimated to cost the industry 2.5 billion [NOK] in direct delousing costs and 7 billion [NOK] through direct and indirect costs, yearly. The salmon louse is still a large problem for the industry and has been the main focus in this thesis.

In order to develop solutions to the salmon louse problem, it is important to understand the very nature of the problem. Most of the work put down in this thesis has been devoted to the section describing the salmon louse and the methods that have been developed against it
The lepeptheirus salmonis molts through eight stages, where its reproduction and host attachment stage is of extra interest. The many methods developed to combat the salmon louse have been categorized through its usage, constraints and applicability.

Through the gained knowledge of the lepeptheirus salmonis and available delousing measures, a simulation model has been developed. The simulation model has as its purpose to estimate salmon louse abundances arising from copepodite pressures generated at external sites, and estimate the effects from using the different delousing methods at hand. The model is constructed in a generic manner, readily enabling implementation of fish farm sites and vessels with delousing capabilities. The simulation model seeks to generate information which will help salmon production companies in their decision making in regards to coping with the salmon louse. Through estimating the effect and consequences of their planned measures on a short and long term basis. It also works as a delousing methodology manual which presents the applicability and constraints of the different delousing measures available in an objective fashion, suited for their specific location.

Keyword:

- Salmon louse
- Delousing methods
- Decision support system (DSS)

Advisor:

Bjørn Egil Asbjørnslett

Address:
NTNU
Department of Marine Technology
N-7491 Trondheim

Location
Marinteknisk Senter
O. Nielsens vei 10

Tel. +47 73 595501
Fax +47 73 595697

Preliminary project definition

NTNU Trondheim
Norwegian University of Science and Technology
Department of Marine Technology

PROJECT THESIS IN MARINE TECHNOLOGY

AUTUMN 2016

For stud.techn.
Erik Andreas Næstvold

Preliminary thesis title**: Delousing methods, constraints and applicability.**

Background

Today Salmon louse is the biggest constraint in Norwegian Atlantic salmon production. The Salmon louse is being resistant to more and more of the delousing methods, as the cheapest method has a habit of being over-used over a longer period of time until it does not have sufficient efficiency. First then the salmon producer heads towards the more expensive method in order to delouse. This in turn leads to several locations with louse being resistant to several delousing methods.

Salmon louse has co-existed for a long time together with the wild stocks of Atlantic salmon. However, with the large densities of salmon present at salmon production facilities the possibilities for massively increased salmon louse exposure has increased dramatically. This again is proven to affect the wild stocks in a negative manner. There are almost no coastal waters along the Norwegian coast that does not have some forecasted copepodites present at any given time. Mainly due to the many “infected” salmon facilities which then expose surrounding waters with salmon louse through the ocean currents.

The salmon louse problem has to date been viewed as a local problem, where each farm site has the responsibility to maintain a low level of salmon louse. This focus neglects the very nature of the salmon louse and how it spreads. Newer research has concluded that salmon louse rises from copepodites pressure developed at external locations to the farm site. Which emphasizes the importance of treating the problem louse problem regionally and not locally as per date.

Objective

Objective of this thesis is to get a thorough overview of the current different active delousing methods being used and that are under development. Categorize pros and cons of the different methods, and perceive by analysis of available data if some methods are more expedient than others at different conditions. Passive delousing methods shall be stated at a more general level.

The thesis shall be the stepping stone for the master thesis where the data shall be utilized in order to develop a sustainable delousing system. Which will emphasize the importance of threating the salmon problem as a regional rather than single location vise system. Which may result in showing the importance of a planned rotational delousing system, active delousing methods vise, on a regional level. Together with subsequent use of passive/preventive delousing systems in order to create a sustainable mariculture of Atlantic Salmon in Norway.

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Norwegian University of Science and Technology
Department of Marine Technology

Tasks

The candidate shall/is recommended to cover the following tasks in the project thesis:

a. Review state of art within the topic of salmon louse and delousing methods. A thorough literature study and present findings in a straightforwardly manner.

b. Explain the biology of the salmon louse, its lifecycle, how the different life phases affect the salmon differently. And present the current copepodites pressure simulator, and its importance to the salmon industry.

c. Every single active delousing method shall be investigated and analyzed. Reason for resistance gained shall to what extent possible with available resources be mapped for each distinct delousing method.

d. See every delousing method active as passive in an overall perspective, where these methods work as tools which should be utilized at its appropriate time. Not only at one site at the time, but also in context to the region it is located.

General

In the thesis the candidate shall present his personal contribution to the resolution of a problem within the scope of the thesis work.

Theories and conclusions should be based on a relevant methodological foundation that through mathematical derivations and/or logical reasoning identify the various steps in the deduction.

The candidate should utilize the existing possibilities for obtaining relevant literature.

The thesis should be organized in a rational manner to give a clear statement of assumptions, data, results, assessments, and conclusions. The text should be brief and to the point, with a clear language. Telegraphic language should be avoided.

The thesis shall contain the following elements: A text defining the scope, preface, list of contents, summary, main body of thesis, conclusions with recommendations for further work, list of symbols and acronyms, reference and (optional) appendices. All figures, tables and equations shall be numerated.

The supervisor may require that the candidate, in an early stage of the work, present a written plan for the completion of the work.
The original contribution of the candidate and material taken from other sources shall be clearly defined. Work from other sources shall be properly referenced using an acknowledged referencing system.

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Deliverable

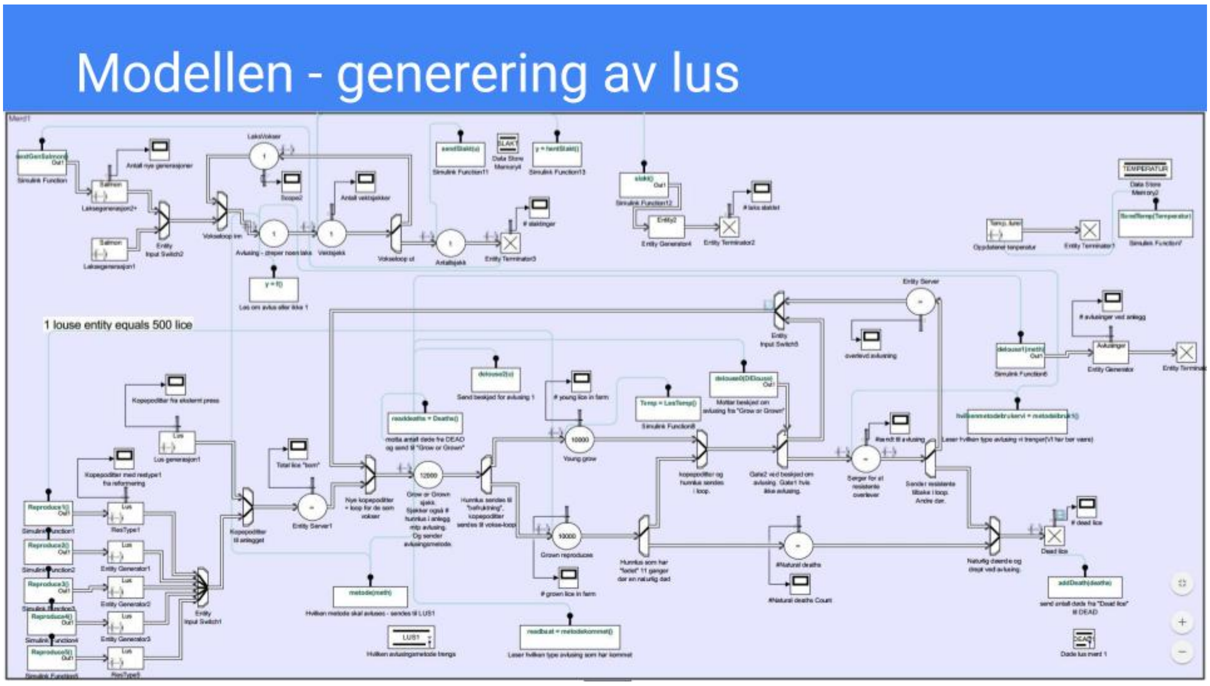
- The thesis shall be submitted in two (2) copies:
- Signed by the candidate
- The text defining the scope included
- In bound volume(s)
- Drawings and/or computer prints that cannot be bound should be organized in a separate folder.
- The bound volume shall be accompanied by a CD or DVD containing the written thesis in Word or PDF format. In case computer programs have been made as part of the thesis work, the source code shall be included. In case of experimental work, the experimental results shall be included in a suitable electronic format.

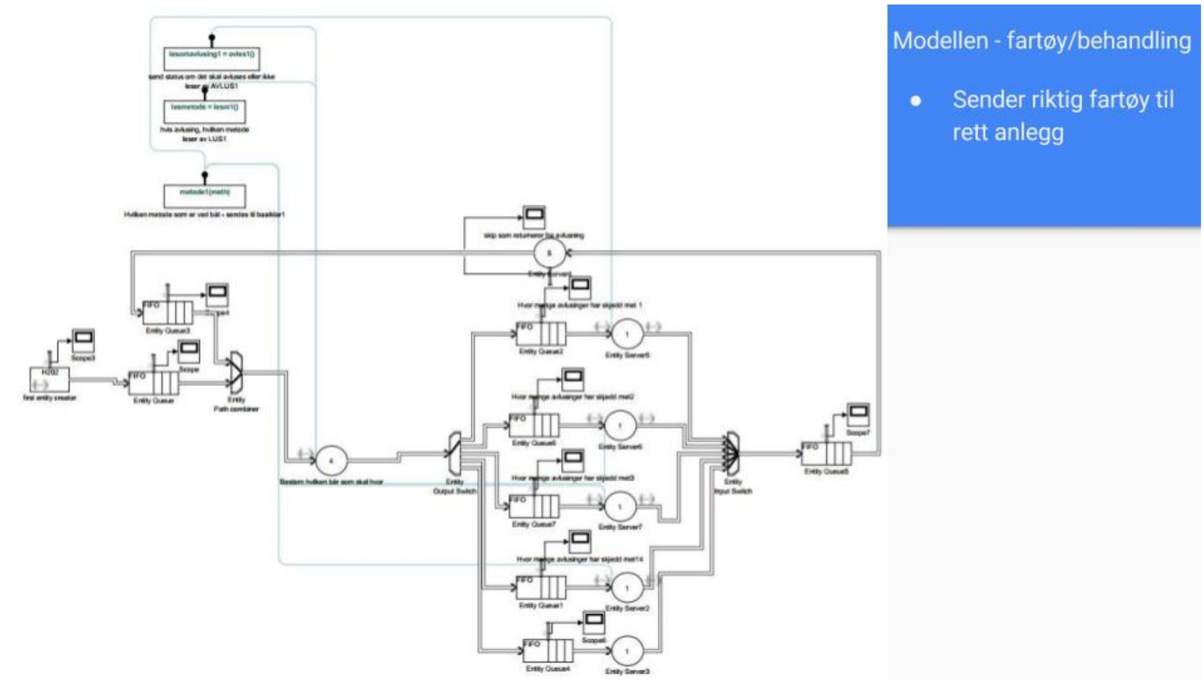
Supervision:
Main supervisor: Bjørn Egil Asbjørnslett.

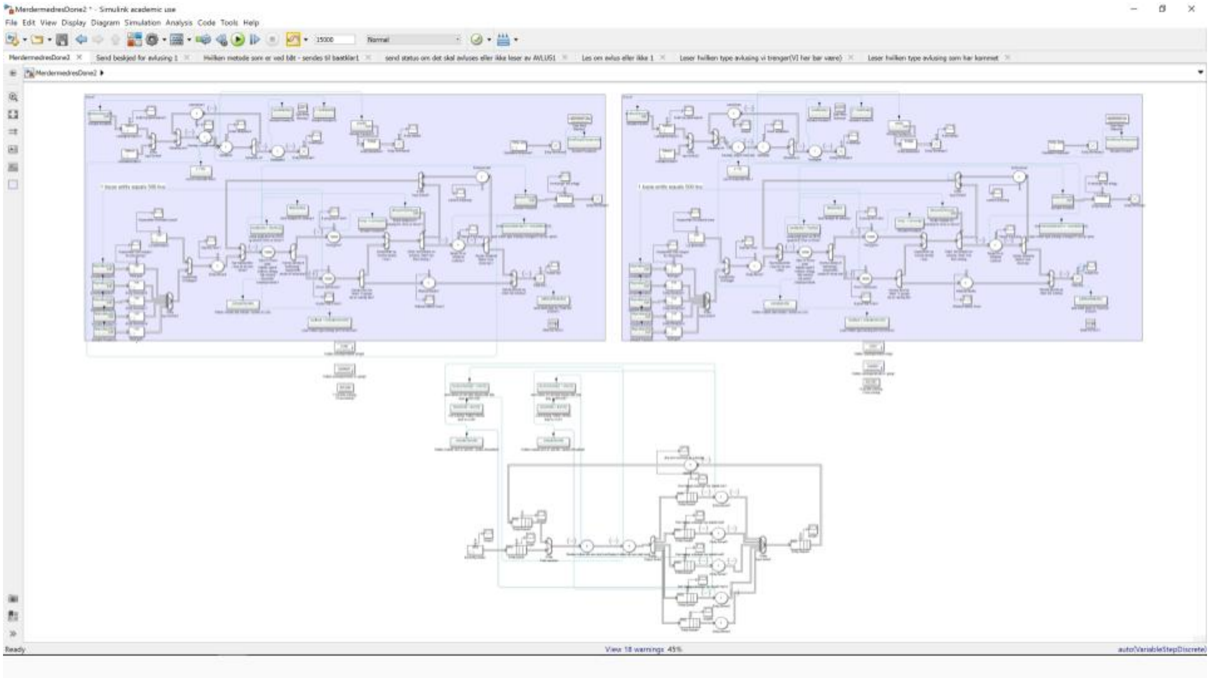
Company contact: Elvin Bugge Aquatic Concept Group.

Deadline: 16.12.2016

C Simulation model



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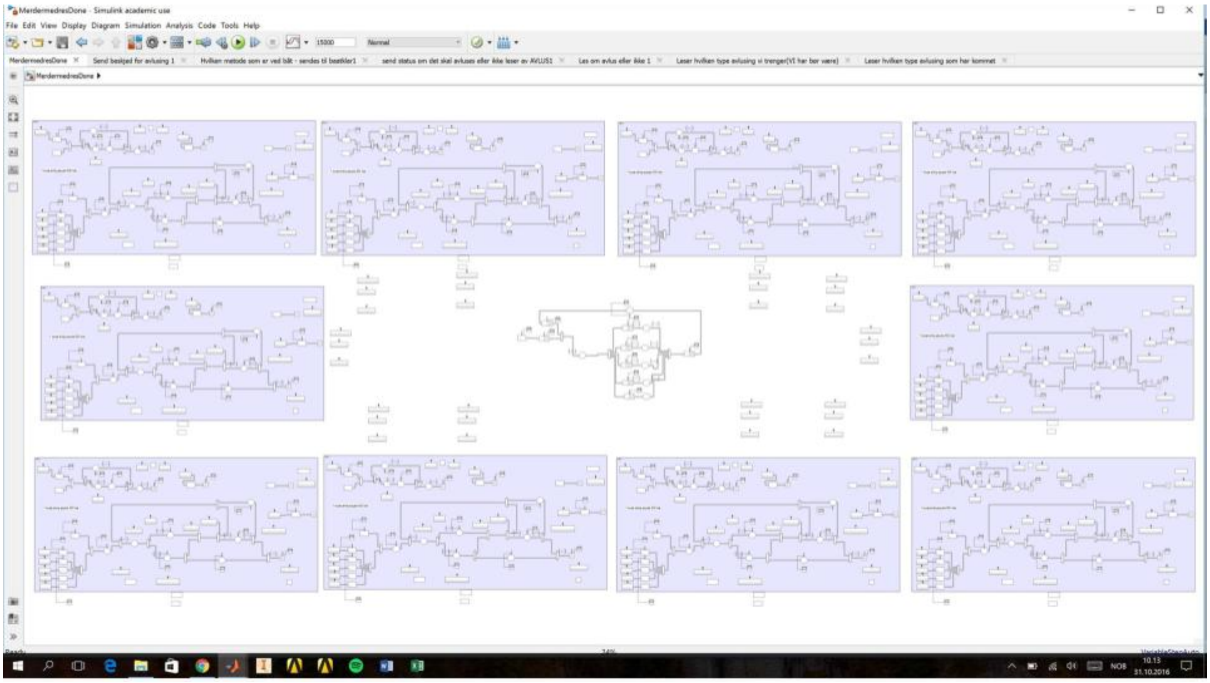
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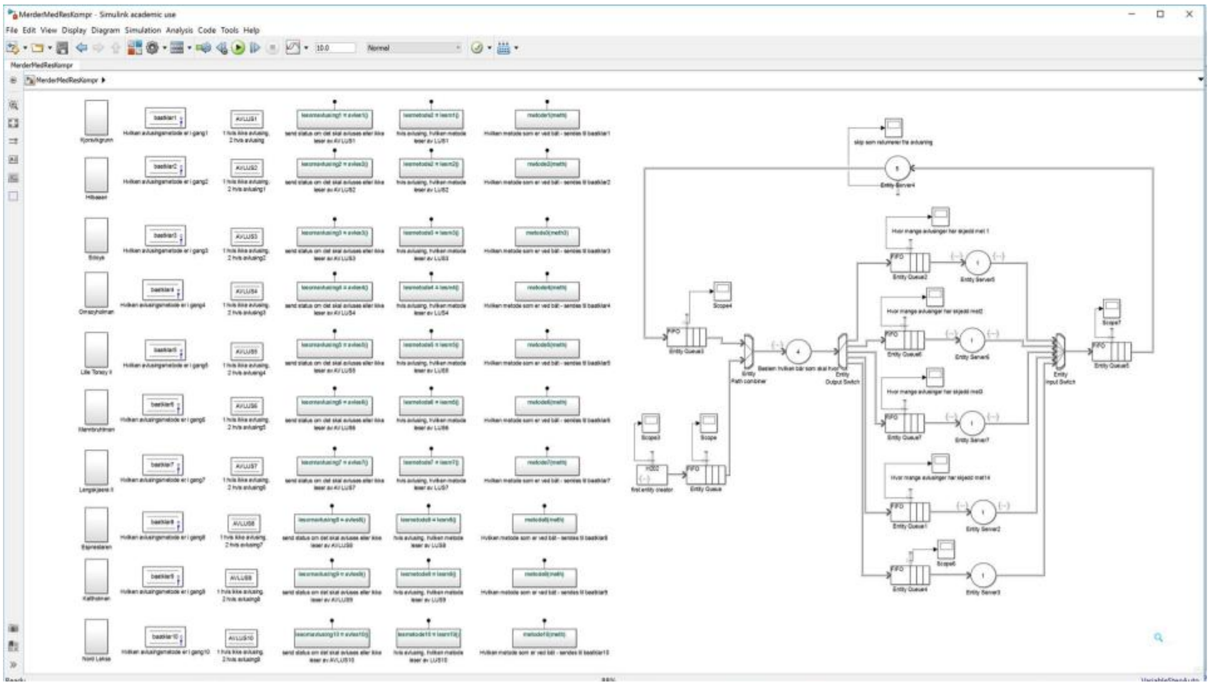
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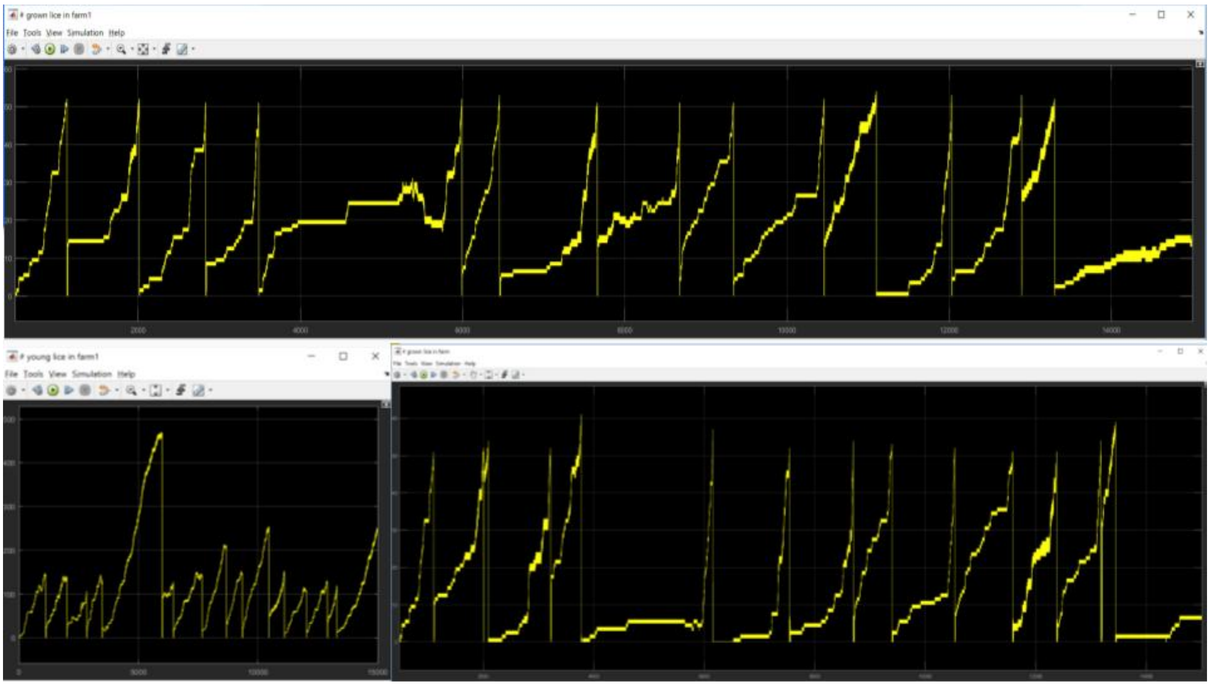
D Simulation results



The graphs illustrate abundances of adult salmon louse for farm one (top), pre adult salmon louse farm one (bottom left) and adult salmon louse in farm two (bottom right). The rapid decrease is due to active delousing methods being utilised. The low abundance development for adult salmon louse seen is mainly due to seasonality (winter, low temperatures and corresponding low development rate).

E Bath treatment procedure

Bath treatment operation with tarpaulin

This describes the process and equipment required to delouse one salmon cage by bath treatment with whole tarpaulin.

Special conditions/constraints

Limitations regarding weather must be decided for each specific salmon farm. It has been found that delousing in whole tarpaulin should not be conducted when the current is stronger than 35 cm/sec due to the deformation of the tarpaulin.

Equipment

Ensure that the designated tarpaulin is water tight with right dimensions for the cage to be deloused. A good way to ensure a good fit for the tarpaulin is to mark it with different diameters, which makes it easy to obtain desirable tarpaulin volume. The tarpaulin volume must be bigger and minimum correspond with the volume of the penetrated cage. (Sett inn bilde av en rund presenning med forskjellige fargekoder samsvarende med diameter). The tarpaulin must have at least ten ropes attached. Four ropes to be used in the direction of the current, and three on each side to ensure a fully enclosed tarpaulin. The applicability of the ropes will be further described in the execution part.

There should be at least two boats and preferably four boats during the operation. Each boat with the possibility of raising the bottom ring? in a coordinated manner. Minimum two of the boats should have crane as these two are installing the tarpaulin. The boat with the tarpaulin should have a crane with triplex for easier handling of the tarpaulin. Snatch block (kasteblokk, reduserer hastighet, men øker laste kapasiteten/forlengelse av kran vinsj.) Is suitable to use if some of the boats does not have sufficient winch/crane capacity for the operation. Oxygen sensors for continuously surveillance of oxygen levels inside the tarpaulin. Reserve tarpaulin and oxygen hoses.

Oxygen hoses and delousing agent equipment uipment:

Salmon health aspects: It is important to have an idea of the salmon's current health status, especially regarding heart-and gill health before delousing. As the delousing process is posing stress onto the salmon, and with a poor health status may and has many times lead to loss of salmon. The salmon must also have been sufficiently starved, approximately between 2-4 days is recommended. The starvation leads to reduced excrements when the tarpaulin is mounted which can lead to poor conditions. The salmon also requires less oxygen when it has no food to process.

Operation Execution

The operation can be divided into three parts: Initialization, execution and de-mounting and completion.

Initialization

The initialization process is every action which has to be completed before one can start the delousing process. The current has to be identified and the boats must be in position accordingly around the cage. The bottom ring must be raised to approximately 6 [m] below surface to create a desirable volume. The tarpaulin shall be mounted by submerging it on the furthermost end of the cage relative to the current, always work against the current to ensure easy handling and appropriate mounting.

After the tarpaulin has been mounted and appropriately sealed oxygen suppliers such as hoses shall be laid out inside the cage. Make sure the cables are spaced neatly to ensure a mean oxygen coverage for the salmon. Lay out the delousing agent hoses in same manner as the oxygen hoses, this is vital as poorly spaced or laid cables may result in delousing agent hot spots. One must also lay out spaced sample hoses to effectively measure the agent concentration from different locations in the enclosed bath. It is important to know the closed volume of the bath in order to inject appropriate amount of delousing agent to achieve the optimum treatment strength. When using hydrogen peroxide the volume is found by injecting a certain amount of hydrogen peroxide into the bath. Take samples from the bath, analyse strength and then by formula one have the enclosed bath volume.

Execution

The dosing equipment should be suited for the specific agent being used as some equipment may not be fit for all delousing agents and vice versa. The dosing hoses should be constructed to dose both vertically and horizontally as vertically dosing has been shown to have a quicker and more even distribution than surface dosing alone. This is easily done by perforating four equally spaced locations on the hose per perforation. Be careful when perforating the hose as the pressure from the dosing equipment must be accounted for in order to ensure that all perforations along the hose emit the same amount of delousing agent.

There are different dosing principals for each of the delousing agents, the following are the only aspects that differ from the bath treatment process as whole.

Pyretroids should be mixed into seawater immediately before out dosing the agent.

Azamethiphos should be mixed into freshwater on the day of delousing. Maximum 48 hours before estimated delousing execution. The agent must be neatly stirred in the freshwater solution for approximately five minutes. When execution is about to commence the freshwater delousing agent should be mixed with 200 [litres] of seawater, then neatly stirred again for five minutes. This solution is then ready for out dosing.

Hydrogen peroxide is most likely to be stored in concentrations of 50% hydrogen peroxide. This high concentration is extremely oxidizing and must be reduced to concentrations below 10%, however in order to achieve a concentration of 2000-2500 ppm in the bath, large quantities of hydrogen peroxide must be added. And during the mixing large quantities of seawater is added. This must be accounted for when estimating required amount of hydrogen peroxide to be added to achieve the desired concentration.

The duration of the delousing agents' exposure to the fish varies from 15-30min depending on the agent in use. The bath treatment timer starts when all of the delousing agent is in the treatment bath.

During the execution oxygen levels, fish behaviour, tarpaulin shape and agent concentration must be continuously monitored. Fish behaviour should be the governing factor for the general health situation for the salmon during the bath treatment. Salmon that shows deviating behaviour as panic, fainting and drastically decreased movement are all lethal signs that must be taken seriously. With quantities of salmon showing the same deviating behaviour as previously explained actions must be taken. The tarpaulin must be removed immediately and the treatment water must be as quickly as possible replaced by fresh sea water. This is most efficiently done by cutting the tarpaulin ropes and the boat with the triplex crane starts rolling in the tarpaulin. In order for quick bath water replacement it is recommended to use one or more of the boats propellers to generate a strong current into the cage. Beware as the propeller may suck the cage net into its propeller generating a much worse situation. The penetrated net should also be expanded as quickly as possible as some of the delousing agents can react with particles in the newly introduced surrounding water forming poisonous solutions. This is especially important when using hydrogen peroxide, as particles such as iron and copper form poisonous gas solutions in contact with hydrogen peroxide which may be present in the sea water. However, this is under discussion as some claim that expanding the net too rapidly may cause panic reaction where the fish swim down and press the bottom of the net. On the other hand, during my stay at MS Urter this panic reaction was observed the second the tarpaulin was removed and the net was still penetrated. (seen in pic...) Regardless this reaction is not wishful as large quantities of salmon may die from pressure damage.

De-mounting and completion

When the treatment time is done, the first step is to remove the tarpaulin. The tarpaulin shall be removed in reverse order as it was mounted. When the tarpaulin is removed the penetrated net should be expanded, first lower the bottom ring then release the net so it may expand to its natural shape. When this is done the sampler and dosing cables shall be pulled out from the cage. When all equipment is de-mounted the oxygen cables and sensors may be retrieved. Note the oxygen supply shall not be turned off before all the cables are out of the water.

Note: A ROV should be utilized during the whole operation to give a visual status of penetrated net shape, tarpaulin launch and deploy and during tarpaulin release. The ROV should be monitoring after the de-mounting to observe fish behaviour. In some cases, large quantities of fish die after the operation and will be easily observed by the ROV. This requires dead fish handling and removal as the amounts of dead fish can be significant.

F Script for estimating enclosed volume of tarpaulin

```
function [Volume,depth]=volumedecider(readtarpaulincircumference,Cagecicumference)
%readtarpaulincircumference=200;
%Cagecicumference=160;
rtc=readtarpaulincircumference;
Cc=Cagecicumference;

t=rtc/(2*pi); %tarpaulin radius
a=Cc/(2*pi); % cage radius
Starpaulin=pi*t^2;
% sjekk om posen er oblate, prolate eller kule
Scheck=4*pi*0.5*a^2; % kule surface

if Scheck>Starpaulin % oblate form
c=a-0.0001; %initial guess of tarpaulin depth
e=sqrt(1-(c^2/a^2));
Soblate=pi*(a^2)*(1+((1-e^2)/e)*atanh(e)); %half oblate

while abs(Soblate-Starpaulin) > 1 && c<a

e=sqrt(1-(c^2/a^2));
Soblate=pi*(a^2)*(1+((1-e^2)/e)*atanh(e)); %half oblate
    c=c-0.001;

end
%---------------Check for prolate form, and initiate calculation----
elseif Scheck<Starpaulin % prolate form
    % If not neatly penetrated the shape may be prolate
    c=a+0.0001;
    e=sqrt(1-(a^2/c^2));
    Soblate=pi*(a^2)*(1+(c/(a*e))*asin(e)); %half sphere
while abs(Soblate-Starpaulin) > 1 && c>a

    c=c+0.001;
    e=sqrt(1-(a^2/c^2));
    Soblate=pi*(a^2)*(1+(c/(a*e))*asin(e)); %half sphere

end
%---------------------------------------------------------------------
%---------------If neither of two, it is a perfect sphere--------
else
    Soblate=Scheck;
end
%-------------------------------------------------------------

depth=c;
Volume=(4/3)*a^2*c;

%end
```