Norwegian University of Life Sciences
Department of Food Safety and Infection Biology
Faculty of Veterinary Medicine

Philosophiae Doctor (PhD)
Thesis 2019:48

Production of Atlantic salmon (*Salmo salar*) in closed confinement systems (CCS) - salmon lice, growth rates, mortality and fish welfare

Oppdrett av atlantisk laks (*Salmo salar*) i lukkede merder
– forekomst av lakselus, vekst, dødelighet og fiskevelferd

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To my children

The true delight is in the finding out rather than in the knowing.

- Isaac Asimov

Table of contents

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This thesis is based on development and testing of closed cages (CCS). These technological farming projects were designed and performed by the commercial partners, while NVI coordinated the research activities on fish health and welfare. It could never have been completed without the enthusiasm and hard work committed by all the great people who were involved in these projects. Most of all I wish to express my gratitude to Anders Næss for the creativity and efforts needed to realize all these projects and to Trond Otto Johnsen for being an indispensable, hardworking and always optimistic jack of all trades.

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List of papers

Paper I

Effective protection against sea lice during the production of Atlantic salmon in floating enclosures

Authors: Arve Nilsen, Kristoffer Vale Nielsen, Eirik Biering, Asbjørn Bergheim

Published: Aquaculture 466 (2017) 41-50

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Paper II

Performance of post-smolt Atlantic salmon in closed confinement systems; Growth, mortality and fish welfare

Authors: Arve Nilsen, Kristoffer Vale Nielsen, Asbjørn Bergheim

Submitted: Aquaculture 2019.03.27

Paper III

The impact of production intensity on water quality in oxygen enriched, floating enclosures for post-smolt salmon culture

Authors: Arve Nilsen, Kristoffer Vale Nielsen, Anders Næss, Asbjørn Bergheim

Published: Aquacultural Engineering 78 (2017) 221-227

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Paper IV

The importance of exercise: Increased water velocity improves growth of Atlantic salmon in closed cages

Authors: Arve Nilsen, Ørjan Hagen, Chris Andre Johnsen, Halvor Prytz, Bingfei Zhou, Kristoffer Vale Nielsen, Marit Bjørnevik

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Summary

Farming of Atlantic salmon have been in rapid growth since the 1970's and is now an important industry in many countries around the North Atlantic as well as Chile. Since 2000, all countries with farming of Atlantic salmon and rainbow have faced challenges with the development of drug resistant sea lice. The use of drugs and the cost of production has increased, this has undermined the aquaculture industry's profitability and reputation. In Norway, the rapid growth of salmon farming was arrested from 2012, mainly because of increasing problems with drug resistant salmon lice (*Lepeophtheirus salmonis*). Norwegian authorities have issued new farming licenses with the purpose of encouraging fish farming companies to solve the most important environmental challenges. Increased production of salmon in land-based facilities, the development of offshore aquaculture and different varieties of closed containment systems (CCS) have been proposed.

When this thesis was developed (2012-2015), the knowledge about fish health and welfare in commercial scale CCS was limited. It was important to assess if the use of untreated deep water could provide sufficient protection against sea lice and if introduction of lice could lead to sea lice reproduction and sustained infestations in the closed cages. Furthermore, it was necessary to investigate growth rates, mortality rates, mortality causes and fish welfare in CCS.

Our studies showed that CCS with water intake at a 25 m depth provided effective protection against sea lice copepodites (*Lepeophtheirus salmonis* and *Caligus elongatus*). Adult *Caligus elongatus* were observed occasionally and at low abundancies. When sea lice were introduced into CCS, we observed no signs of reproduction or sustained infestations. Without sea lice, there was no need for treatments. This reduced the environmental impact and improved fish welfare with production of salmon in CCS, compared to traditional net-pens.

Mean thermal growth coefficient (TGC) for post-smolt in CCS was close to 3.0, ranging between 2.24 and 3.94. The lowest growth rates were caused by low specific flow and suboptimal water quality (early trials). For the majority of cages, increased water velocity could be an important explanation variable for increased growth rates and condition factors in CCS, compared to net-pens. Increased water temperatures during winter (September-May) in CCS compared to net-pens could also be of significance,

because most trials were performed with off-season smolt (S0). Results from large scale trials were supported by small scale trials with post-smolt, where moderate water velocities (19-21 cm/s) showed a significant increase of growth rates and condition factor compared to low water velocities (6-8 cm/s). Increased water velocities increased the fillet yield in harvest sized salmon (3000 g), but without increased deposition of body fat.

Cumulated mortality rates in CCS were moderate to low, compared to mortality rates in net-pen studies. Ulcers and fin rot caused by bacterial infections was an important health and welfare issue during the post-smolt period and occurred at different fish sizes and different water temperatures. Simultaneous lesions and bacterial infections of the skin on the body and the fins are probably caused by the same bacterial pathogens. These chronic infections caused increased mortality rates, but were also associated to suppressed appetite, reduced growth rates and condition factors. Suboptimal quality of smolt at sea transfer increased the risk of post-sea-transfer mortalities.

From our studies, recommended minimum specific water consumption (SWC) during production of post-smolt Atlantic salmon is 0.2 L/kg/min. The recommended maximum feed load is 35-40 g feed/m^3. Mean values of oxygen saturation were close to the values described as optimal for the growth performance of farmed Atlantic salmon. The (short-term) extreme variations were above the threshold for severe hypoxia (LOC) and below toxic levels. Carbon dioxide (CO_2) concentrations were mostly below the threshold for negative impact on welfare and growth performance (10 mg/L), with a few cases of CO_2>15 mg/L, where we also observed a negative impact on appetite and welfare. Harmful levels of ammonia (NH_3) were not recorded.

All threshold values and indications of low mortality and high growth rates should however be interpreted with caution. Our understanding of the complex interactions between the salmon, the environment and the rearing conditions is still limited. Future research should not aim narrowly at identifying the maximal biological input and minimum standards of life conditions for the farmed fish. It should be equally important to study how farmed salmon respond to more optimized and high-quality environments, in both closed cages and other rearing systems.

Sammendrag

Oppdrett av laks har vært i rask vekst siden 1970-tallet og er nå en viktig næring i mange land rundt Nord-Atlanteren samt Chile. Siden 2000 har utvikling av legemiddelresistente lakselus ført til store utfordringer i alle land med oppdrett av Atlantisk laks og regnbueørret. Mengden av legemidler og kostnadene til produksjon har økt og dette har undergravd oppdrettsnæringens lønnsomhet og omdømme. I Norge stagnerte produksjonsveksten i 2012, hovedsakelig på grunn av problemene med lakselus (*Lepeophtheirus salmonis*). Myndigheter og næringsliv i Norge har satt som mål å utvikle ny oppdrettsteknologi for å løse noen av disse utfordringene. Økt produksjon av laks i land-baserte anlegg, utvikling av offshore merdanlegg og ulike varianter av lukkede merdsystemer i sjø er foreslått.

Da arbeidet med denne avhandlingen begynte (2012-2015) var det lite tilgjengelig kunnskap om fiskehelse og velferd ved drift i lukkede merdsystemer. Det var viktig å finne ut om bruk av urenset dypvann kunne beskytte mot lus og om inntak av små mengder lus i lukkede merder kunne føre til oppformering av parasittene og høye lusetall på laksen inne i merdene. Det var også viktig å kartlegge dødelighet og dødelighetsårsaker, veksthastighet og fiskevelferd ved drift av lukkede merder.

Vi fant at lukkede merder med inntak av urenset vann fra 25 meters dyp ga fullgod beskyttelse mot påslag av luselarver (både *Lepeophtheirus salmonis* og *Caligus elongatus*). Voksne skottelus (*C. elongatus*) ble påvist sporadisk og med et lavt antall lus per laks. Hvis vi slapp inn små mengder lus klarte parasittene ikke å oppformere seg på laksen inne i de lukkede merdene. Uten lus ble det heller ikke noe behov for behandlinger. Dette er en viktig gevinst både for miljø og fiskevelferd.

Gjennomsnittlig vekstrate (TGC) for post-smolt i lukkede merder var nær 3.0, med variasjon mellom 2.24 og 3.94. Dårligst veksthastighet fikk vi i merder (tidlige forsøk) med lav vannutskifting og suboptimal vannkvalitet. For de fleste merdene ble økt vannhastighet sett som en sannsynlig årsak til økt vekst og høyere kondisjonsfaktor hos laks i lukkede merder, sammenlignet med laks i åpne merder. En høyere vanntemperatur i lukkede merder om vinteren (september – mai) hadde sannsynligvis også en betydning fordi de fleste forsøkene ble gjort med nullåring (S0). Det vi fant i de store merdene ble støttet av resultater fra småskalaforsøk med post-smolt hvor moderat vannhastighet (19-21 cm/s) ga en signifikant økt vekst og kondisjonsfaktor

sammenlignet med lav vannhastighet (6-8 cm/s). Økt vannhastighet ga også økt filetutbytte på stor fisk (3000 g), men uten noen økning i fettprosent.

Samlet dødelighet i lukkede merder var moderat til lav, sammenlignet med dødelighetstall fra åpne merder. Sår og finneråte ble forårsaket av bakterieinfeksjoner, var en utfordring for fiskehelse og fiskevelferd gjennom hele produksjonsperioden og kunne opptre ved ulike størrelser av fisken og ved ulike vanntemperaturer. Hudsår og finneråte utviklet seg som regel samtidig og så ut til å skyldes den samme typen bakterielle infeksjoner. Sår og finneråte forekom som kroniske infeksjoner der økt dødelighet også var fulgt av redusert matlyst, nedsatt vekst og lavere kondisjonsfaktor. Vi så også at dårlig smoltkvalitet ga økt risiko for dødelighet den første tida etter sjøsetting.

Våre studier viser en nedre grense for spesifikt vannforbruk på 0,2 L/kg/min ved produksjon av post-smolt laks i lukkede merder. Anbefalt øvre grense for fôring har vi beregnet til 35-40 g fôr/m^3 tilført vann. Gjennomsnittlige oksygenverdier i lukkede merder var nær de verdiene som beskrives som optimale for oppdrett av atlantisk laks. De kortvarige variasjonene var større, men de laveste registrerte oksygenverdiene var over grensen for alvorlig hypoksi (LOS) og de høyeste oksygenverdiene var lavere enn det som er kjent å kunne gi skade på fisken. Nivåene av karbondioksyd (CO_2) var for det meste under kjente maksimumsgrenser (10 mg/L). Ved noen tilfeller steg nivået av CO_2 over 15 mg/L, og da så vi som regel en negativ effekt på vekst og velferd. Skadelige nivåer av ammoniakk (NH_3) ble ikke påvist.

Det er viktig å tolke alle slike grenseverdier og beskrivelser av dødelighet og tilvekst med forsiktighet. Vår forståelse av den kompliserte sammenhengen mellom fisk, miljø og forholdene inne i merdene er ennå begrenset. Videre forskning bør ikke fokusere utelukkende på hvordan oppdrett kan drives med maksimal biologisk belastning og med minimumsstandarder for fiskens miljø. Det bør være like viktig å kartlegge hvordan laksen kan ha det og hvordan den kan prestere hvis den tilbys så optimale miljøforhold som mulig, enten det er i lukkede eller i åpne merder.

1. Introduction

1.1 Salmon farming in Norway

Aquaculture is defined as the culturing of plants and animals in fresh, brackish and marine waters. In 2016, the global aquaculture production of fish, crustaceans, molluscs, plants and other aquatic organisms was 110,208,218 tons (FAO, 2018). According to FAO statistics, more than 92% of the world's aquaculture production takes part in Asia, with China, Indonesia, India and Vietnam as some of the most important countries. The majority of aquaculture products come from freshwater fish (42%) and plants (27%). In 2016, the global production of salmonid fish was 3,319,715 tons, with Atlantic salmon (2,247,759 tons) and rainbow trout (814,091 tons) as the principal species. In Europe, diadromous fish (such as salmonids) and molluscs are the most important products. In Norway, Atlantic salmon and rainbow trout are dominant (99.7% of Norway's total aquaculture production in 2017). Norwegian production of Atlantic salmon increased from 4,312 tons in 1980 to 1,236,619 tons in 2017, close to 50% of the global production, with smaller volumes of rainbow trout and with a rapid increase in numbers of cleaner fish since 2008 (Norwegian Directorate of Fisheries, 2018a,b) (Figure 1).

Salmon and rainbow trout farming is usually divided into a freshwater period and a seawater period. In land-based hatcheries with freshwater, the fish grow until they have reached full seawater tolerance at approximately 100 grams. The on-growing period until harvest takes place in open net-pens located at sea sites along the coast. Freshwater production systems were established as pond-systems of different sophistication around the world in pre-industrial time (Beveridge and Little, 2002). Open net-pens located in a large recipient (the ocean) has been necessary provide a physiological environment suited to the rapid growth of anadromous fish such as salmonids. In Norway, cages with live fish were probably first used by fishermen to hold live fish of different species until they were ready for sale, but the first commercial sea cages for salmon production in Norway were designed and tested in 1970 (Tvenning, 1991). In 1973, the authorities established a system of geographically distributed production licenses with a maximum cage volume of 12,000 m^3 per license (Gjedrem, 1993). Growth was slow until the mid-80s, when the introduction of new and improved farming technology made it possible to expand cage volumes and increase stocking

numbers. This growth was further fuelled in 1985 when the authorities handed out free licenses to land-based hatcheries for the production of smolt (Tvenning, 1991).On average, the production capacity of hatcheries and smolt farms increased 20 times between 1985 and 2000 (Bergheim, 2009). Due to the abundance of available smolt, new and larger cages, better site infrastructure, improved feed quality and faster-growing fish, production at the sea sites increased tremendously (Asche et al., 2013).

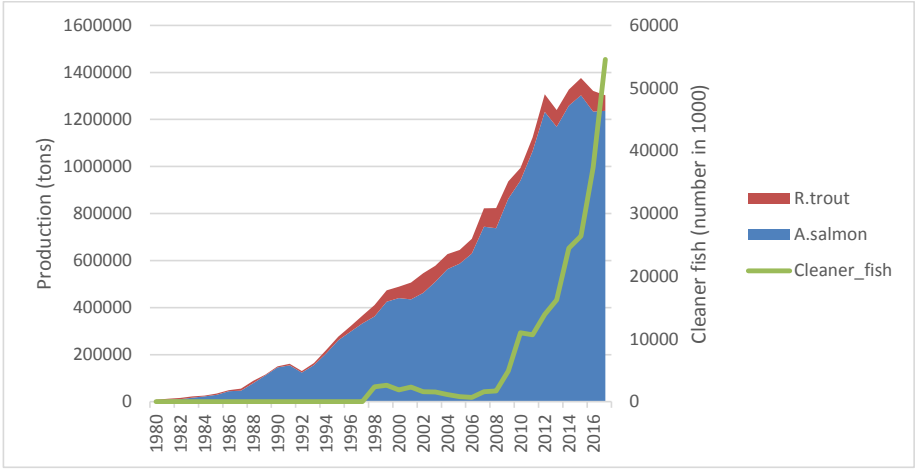

Figure 1. Left axis: production of Atlantic salmon and rainbow trout (tons), right axis: use of cleaner fish (numbers in 1000), Norway, 1980-2017 (Source: Norwegian Directorate of Fisheries, 2018).

1.2 BIG is beautiful

I started my practice as a 'fish doctor' in 1995. IOver the years, I visited a diversity of fish farms in the southern part of the county of Nordland. The fist years they were still using the small, homemade sea cages constructed from traditional fishing nets suspended from floating systems made of wood and styrofoam. These relicts soon disappeared and were replaced by new and larger nets with floating, circular plastic rings or rectangular steel cages. New technology transformed salmon farming from a spare time occupation for farmers and teachers into industry and big business. The nets with steel cages were dominant in the fjords, especially in central West Norway, where they often were moored close to the shore with a gangway running from the landbase to the cages. At

the more exposed sites, as in most of northern Norway, circular plastic rings moored with a larger distance between each cage were the preferred technology (Figure 2).

Figure 2. Examples of the new steel cages (left) and circular plastic cages (right) that dominated in Norwegian salmon and rainbow trout farms from 1990 (Photo: Arve Nilsen).

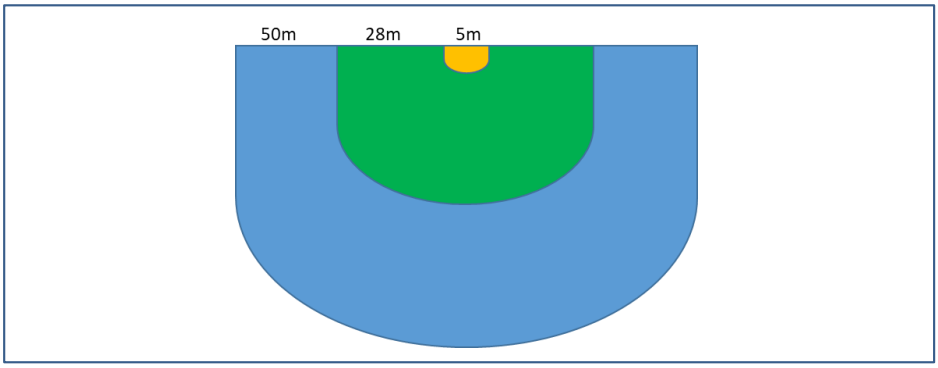

Figure 3. Schematic intersection of pen from 1980 (yellow: 5 m diameter) and 2010 (blue: 50 m diameter). The green globe (28 m diameter) illustrate the size of the CCS with 6000m³ volume described in this thesis.

These cage systems were upgraded step by step until they reached the size used today; i.e. steel cages with 20-40 m sides and circular cages with a 120-157 m circumference and up to 30-50 m deep (Figure 3). The volumes of modern net-pens range from 20,000 to 80,000 m^3 (Oppedal et al., 2011) where the water exchange is driven by the natural coastal or tidal current. This is a cheap and potentially powerful method of water circulation; the total water volume in even the largest net-pens can theoretically be replaced within a few minutes.

Despite the vastness of the Atlantic Ocean, water exchange and water quality is not always optimal in these underwater megastructures filled with live fish. In a flow-through system without oxygenation, depletion of oxygen is the first limiting water quality parameter. Net-pens provide a possibility of rapid volume upscaling in salmon production with a minimum of technological input to safeguard water quality. In my years of practice from 1995 to 2015, even sites with a million fish or more were usually operated with no other environmental supervision than the logging of water temperature at a 3 or 5 m depth. Nonetheless, low water flow and levels of dissolved oxygen (DO measured as % saturation) down to severe hypoxia (30% saturation at 12 ºC) have been recorded in the centre of commercial net-pens (Vigen, 2008; Remen, 2012). The levels of DO have a direct impact on fish growth and feed conversion ratio, (Bergheim et al., 2006; Thorarensen and Farrell, 2011). Recurrent or 'tidal' hypoxia is identified as an important restriction for welfare and productivity at sea sites when stocking density, growth rates and water temperatures are all high (Oppedal et al., 2011). However, other topics besides water quality have often dominated the debate about cage size and the number of fish stocked in each cage or at each sea site.

In Norway, production per license increased from 26 tons in 1980 to 1,130 tons in 2010. At the same time, concentration of ownership increased and large firms expanded (Asche et al., 2013). The availability of new production sites and the increased volumes of fish that was possible to stock in the new and larger cages are two of the most important factors behind this growth. Suboptimal water quality, concern for fish welfare and uncertainty about how to manage such volumes of fish during delousing procedures could be a rationale for limiting the size of cages and total numbers of fish per site. However, it was the fear of accidents and massive episodes with escaped fish that induced the authorities to set a maximum limit for the number of fish allowed to be stocked in any single cage (Norwegian Ministry of Trade, Industry and Fisheries, 2011). This regulation ended the discussion about building net-pens with a diameter larger than 50 m, most of all because larger cages would lead to less intensive use of cage volumes and increased production costs. At the same time, the maximum allowed size for smolt or post-smolt produced at land-based facilities under the regulations of smolt production (free licenses) was increased from 250 to 1000 g (Norwegian Ministry of Trade, Industry and Fisheries, 2011). This was an incentive to boost innovation in land-

based post-smolt production technology, and a response to increasing problems with drug-resistant salmon lice (see 'The sea lice challenge'). Production of larger post-smolt in flow-through (FT) or recycling aquaculture systems (RAS) could shorten the production period in open net-pens. It has been postulated that this could limit sea lice infestation rates and thus reduce the need for treatments. Since 2012, both scientists (DKNVS, 2012) and politicians (Norwegian Ministry of Trade, Industry and Fisheries, 2015) have argued for a five-fold increase in Norwegian salmon production by 2050. Arguments against such growth without first solving basic problems such as diseases and environmental impact have also been forwarded (Alsos, 2018). In the same period, the negative impacts of biological and environmental problems have accumulated. In hindsight, the growth of salmon and trout production in Norway was arrested already in 2012 (Figure 1).

During decades of rapid growth in the salmon farming industry in Norway, the population of wild Atlantic salmon spawning in Norwegian rivers has declined. The anadromous salmon is vulnerable to environmental and ecological changes in both rivers and in the marine habitat where they grow until spawning size, and the precise mechanisms behind this dramatic reduction in population size have been a matter of debate. A report from the Norwegian Scientific Advisory Committee for Atlantic Salmon (Forseth et al., 2018) summarises the situation as follows: *'Escaped farmed salmon, salmon lice and infections from salmon farming are the greatest anthropogenic threats to Norwegian wild salmon. The proportion escaped farmed salmon in the rivers is reduced in recent years, and the risk of further loss of wild salmon due to escaped farmed salmon is reduced from very high to high. The knowledge of infections from salmon farming is poor.'*

In the rest of this introduction, I will discuss what I believe have been the most important reasons for the recent stagnation in Norwegian salmon farming and how this has been an incitement for the development of new farming technologies. I will also describe the fundamental principles of fish welfare studies.

1.3 Diseases as biological constraints

The farming of salmon and rainbow trout combines intensive farming and interactions with the marine environment (Pettersen et al., 2015). After outbreaks at one farm (the index case), infectious agents are easily distributed to adjacent farms via coastal currents, or by vectors like escaped (farmed) or migrating (wild) fish or anthropogenic activities (people). The risk of new epidemics originating from a few index cases is likely to increase with increased production volumes, density of sites and the stocking density of individual sites. The marine ecosystem where aquaculture takes place is also an environment and resource of interest for other private enterprises and for the public. The diseases occurring in fish farming could have a negative external influence due to disease spill over from one farm to another (Kristoffersen et al., 2009; Kristoffersen et al., 2013; Gustafson et al., 2014; Pettersen et al., 2015; Pettersen et al., 2016) or from farmed fish to wild fish populations (Garseth et al., 2013). Treatments against bacterial diseases and parasites using feed antibiotics or pesticides have a possible negative impact on non-target species around the farms (Samuelsen and Agnalt, 2018). Specific diseases, like ISA, also have socio-economic implications by leading to restrictions on the international trade of salmon products (NRK, 2015).

A successful and intensive aquaculture industry will depend on efficient strategies to control transmissible diseases. The main control strategies for transmissible diseases in aquaculture can be defined as (Thrusfield, 2005):

1. Control by attempts to eradicate the infectious agent.
2. Controlling the disease, but living with the infectious agent.
3. No control, but coping with (and trying to minimise) the costs associated with disease.

The two first strategies depend on a close public-private partnership, agreements on cost-sharing protocols and how to allocate property rights of the common-pool resources (Pettersen et al., 2015). If actions taken by the public sector affect the short-term profitability of private businesses, e.g. with depopulation and fallowing strategies, the consensus needed for such a partnership could be undermined. If trust erodes, other strategies will be developed. Lack of basic knowledge about the specific infectious agents, their transmission and survival in the marine environment could make it difficult to reach formal agreements on effective control strategies. Within the industry,

the existence of free-riders could also contribute to situations described as 'the tragedy of the commons', where the short term interests of individual farms or companies violate the long-term interests of all the others sharing the same environment and pathogen reservoir.

Epidemic diseases has been an important constraint to growth of salmon production. Today, farming of Atlantic salmon and rainbow trout takes place in significant quantities in Norway, Chile, Scotland, Canada and the Faroe Islands (Asche et al., 2013; FAO, 2018). In Norway, Chile and the Faroe Islands, salmon farmers have experienced periods of dramatic economic loss caused by disease outbreaks. The most violent episode was the almost total collapse of salmon production in Chile after the outbreak of Infectious Salmon Anaemia (ISA) between 2007 and 2010 (Egidius et al., 1986, Thorud and Djupvik, 1988). In the Faroe Islands, salmon farming became almost extinct in 2006, again because of an outbreak of ISA (Pettersen et al., 2015). Although they also experienced their own share of environmental problems, sea lice and infectious diseases (Brun et al., 2018), Scotland and Canada experienced no obvious collapse in salmon production in the same period (Asche et al., 2013). In Norway, the heaviest impact of infectious diseases on salmon farming occured from 1982 to 1992 during the simultaneous outbreaks of several epidemic bacterial and viral diseases. The combined effects of diseases, a reduced biological output and low market prices were important drivers behind the wave of bankruptcies in Norwegian salmon farming in 1991. After 1991, restructuring of ownership, improved vaccines and reduced production costs were among the most important factors behind a new period of rapid growth. From 1993 onwards, as mentioned earlier, licenses aggregated to larger companies with integrated production from smolt to marketing. In 1997, 70 companies produced 80% of Norwegian salmon; in 2012, this number was reduced to only 20 companies (Asche et al., 2013). Through close cooperation between public authorities, research institutions and the farming industry, relatively cost-effective control strategies for several of the most important diseases have been implemented. The market price for salmon has continued to increase (Norwegian Directorate of Fisheries, 2018a), stimulating increased production capacity until the growth of salmon farming in Norway was arrested from 2012. This was described as a result of the combined effect

of salmon lice, emerging diseases, increased public awareness of negative environmental externalities and implementation of new regulations (Asche et al., 2013).

As mentioned above, diseases had a serious impact on the new farming industry in Norway already during the first period of rapid growth in the early 80's. ISA and cold water vibriosis were first discovered in Norwegian salmon farms (Egidius et al., 1986; Thorud and Djupvik, 1988). Both diseases have subsequently been diagnosed in all other major salmon farming regions in the northern hemisphere (Sørum et al., 1993; Aamelfot et al., 2018). Between 1983 and 1993, the use of antibiotics in Norwegian aquaculture increased faster than the growth of salmon production (Asche et al., 2009). The peak was reached in 1987, with 0.9 g of feed antibiotics used per kg of produced salmon, with furunculosis and cold water vibriosis as the most important diseases in Atlantic salmon and vibriosis (*Vibrio anguillarum*) the most important disease in rainbow trout (Lillehaug et al., 2003; Grave and Brun, 2016).

The prevalence of all these diseases in Norway has been very low in recent years (Hjeltnes et al., 2018), thanks to efficient control measures. Vaccines were developed for cold water vibriosis in 1989 (Lillehaug, 1990) and oil adjuvant vaccines for furunculosis in 1990 (Lillehaug et al., 1992). Infectious pancreas necrosis (IPN) was almost eradicated by implementation of a Quantitative Trait Locus selection (QTL) breeding program. The struggle against ISA has been more arduous, involving relocation and restructuring of farming operations, forced slaughtering and zonal fallowing as the most efficient measures (Vågsholm et al., 1994). By implementing even harsher regulations based on early detection and depopulation, ISA has nearly been eradicated in both Scotland (Stagg, 2003) and the Faroe Islands (Pettersen et al., 2015). However, an increasing number of outbreaks has been recorded for emerging diseases like pancreas disease (PD, Salmonid alphavirus) cardiomyopathy syndrome (CMS, piscine myocarditis virus) and heart and skeletal muscle inflammation (HSMI, piscine ortorheovirus) (Hjeltnes et al., 2019). Pancreas disease is managed as an endemic disease in Scotland, Ireland and Norway, although mortality and biosanitary measures represent heavy losses for the affected farms and the total salmon industry in these countries (McLoughlin and Graham, 2007; Aunsmo et al., 2010). Pancreas disease of salmon was listed by the World Organisation for Animal Health (OIE) in 2013. However, PD has no impact on trade relations and the negative externalities seem mostly to be

confined to the salmon farms at risk of getting the disease. Norwegian salmon farmers, backed by the authorities, established a barrier between endemic and non-endemic areas in 2006. Motivation to sink the short-term costs of depopulation strategies in endemic areas was low. Thus, diseased fish were often fed until harvest size and the infection pressure was allowed to accumulate, with a steadily increasing risk of spill over to new regions. Farms bordering the endemic area were at risk of losing motivation to depopulate their farms to protect the interests of what could be seen as the 'free-riders' downstream. With a new and less virulent serotype (SAV2) spreading rapidly across the old barrier at Hustadvika in 2011 (Johansen et al., 2013), farmers in the new endemic SAV2-area were even less enthusiastic about undertaking aggressive depopulation strategies. Due to a lack of strong public-private partnerships, pancreas disease epidemic (first SAV3 and then SAV2) has been allowed to pick up speed. The same can perhaps be said of the other two emerging viral diseases in Norway; heart and skeletal muscle inflammation (HSMI) and cardiomyopathy syndrome (CMS) (Hjeltnes et al., 2019). However, in the case of these two diseases, lack of knowledge about etiology, epidemiology and virus properties also plays an important role.

An important lesson learned from these disease outbreaks in Norway could be that it is difficult to identify all costs associated with disease control and disease outbreaks. The direct costs associated with disease outbreaks and disease management (e.g. SAV2 and SAV3) in themselves do not seem to constitute a strong enough motivation to establish an effective public-private partnership to implement strict control measures. Or at least, this is not a leading priority as long as the short term operational margins are high, as they have been for salmon production in Norway for the last 15 years or so (Norwegian Directorate of Fisheries, 2018a). However, when strong negative externalities appear (e.g. trade restrictions from ISA, massive use of antibiotics to cure bacterial diseases), motivation for control measures is boosted and epidemic diseases can be reduced to sporadic incidents. For widespread viral diseases like PD, HSMI and CMS there could also be hitherto unknown and negative spill over effects on wild salmonid populations.

1.4 The salmon lice challenge

Compared to all these infectious diseases and contingency measures, salmon lice were for a long period viewed more as a nuisance (in my experience). There were problems with lice at many sea sites, but in the media and in the public mind, salmon lice had no chance of competing with the recent financial turmoil of the aquaculture industry or the many emerging infectious diseases in terms of seriousness. For a while, everybody seemed almost to forget about the salmon lice. In the 2005 fish health report from the Norwegian Veterinary Institute, the information about salmon lice was short and relatively optimistic (Bornø et al., 2006):

> *'Salmon lice now seldom occur in large numbers per salmon in farming facilities. A national action plan to combat salmon lice has been implemented for eight years and appears to have had a good effect. On average there were a lower number of mature female lice in 2005 than in both 2003 and 2004. The proportion of facilities that have treated against salmon lice increased somewhat from 2004, and during the same period the use of wrasse has fallen somewhat. In general, the trend in the last three years has been moving in the direction of a lower number of mature female lice and mobile lice per fish. The large increase in the number of farmed salmon (potential growth organisms) mean that there are still a substantial number of salmon lice in Norwegian farming, with the problems this entails. They primarily represent a problem for wild salmon stocks. Large amounts are spent on medications to treat salmon lice today. A vaccine is being worked on which, if successful, may be important in limiting the problems with salmon lice in the Norwegian farming industry. If successful in reducing the general incidence of infections, this will also benefit wild salmon.'*

Twelve years later, the perception of the salmon lice challenge had changed dramatically, and in the annual health report for 2017, the veterinary institute used 14 pages to analyse the salmon lice situation and the welfare challenges connected to treatments against lice (Helgesen and Jansen, 2018). The situation was summarised as follows:

> *'The injurious effect of salmon lice remains the major fish health-related problem in Norwegian aquaculture. The health- and welfare consequences of salmon louse treatment relates mainly to the acute and often fatal injuries associated with the treatments themselves.'*

This development deserves some attention because it has direct implications for the development of closed containment systems (CCS) described in this study. Returning to the different strategies for the control of infectious diseases in aquaculture, eradicating salmon lice is not an option. With salmon lice, there are defined several important negative externalities, both for the environment and for the commercial interests of all fish farmers sharing the same marine ecosystem. Therefore, before looking at the possibilities and challenges of new cage technologies, I will give a short description of sea lice biology, the control measures implemented against salmon lice, the development of drug-resistant salmon lice and the most important non-medicinal treatment methods.

Parasite biology

Parasitic copepoda (sea lice) infect a wide range of wild and farmed marine fish species, and have been a key constraint to the continued growth of salmonid aquaculture worldwide (Costello, 2009a; Torrissen et al., 2013). In the North Atlantic region, the two crustacean ectoparasite species usually found on salmonids in seawater are *Lepeophtheirus salmonis* and *Caligus elongatus* (Pike and Wadsworth, 1999; Boxaspen and Torrissen, 2013; Torrissen et al., 2013) (Figure 4). In Chile, the sea louse *Caligus rogercresseyi* is one of the major health problems in salmon farming (Bravo, 2003), but sea lice have not been reported as a major salmonid health issue in Australia (Nowak et al., 2011; Helgesen and Marin, 2018). *L. salmonis* is often referred to as the salmon louse because it is specific to salmonids, especially Atlantic salmon (*Salmo salar*). *C. elongatus* has a similar life cycle to *L. salmonis*, but without the mobile stages on the host (Piasecki and Mackinnon, 1995). *C. elongatus* is less host specific, has been collected from 80 different species (Boxaspen, 2006) and aggregation of *C. elongatus* on wild lumpfish along the coast could be an effect of a larger reservoir on ocean-living lumpfish (Heuch et al., 2007). The affinity of *C. elongatus* for lumpfish could also be bad news for salmon farms depending on lumpfish as a prophylactic measure against salmon lice. From northern Norway, this has been reported as a problem, with caged lumpfish dying from skin lesions caused by *C. elongatus* infestations[1]. When abundance exceeded 5 to 10 lice

[1] https://ilaks.no/kunne-i-ekstreme-tilfeller-telle-opp-mot-1000-skottelus-per-fisk/

per lumpfish, medication was often used. For a full description of the life cycle and biology of salmon lice, see the doctoral thesis of Aaen (2016) and the web sites of the Norwegian Veterinary Institute and the Institute of Marine Research [2]. In this thesis I will use the term 'salmon lice' when referring to *L. salmonis*, and 'sea lice' when referring to both species. Sea lice live and reproduce on fish, but spread by the release of egg strings into the seawater. The eggs in these strings hatch and develop into planktonic infective stages (Costello, 2009b; Brooker et al., 2018). Salmon lice accumulate on farmed salmonids and lead to stress, skin lesions and mortality (Nolan et al., 1999; Wagner et al., 2003). Salmon lice originating from salmon (and trout) farms are considered a cause of increased mortality in wild salmonid populations and a threat to the environmental credibility of salmon farming (Costello, 2009c; Torrissen et al., 2013; Karlsen et al., 2018a).

Figure 4. From left: Adult female Caligus elongatus, adult male Lepeophtheirus salmonis and adult female Lepeophtheirus salmonis (without egg strings) (Photo: A. Nilsen).

Control strategy

A key concept in theoretical epidemiology is how increasing host density promotes the population growth of a parasite because the chances of finding a host increase with host density (Thrushfield, 2005). As expected, increased densities of farmed salmon lead to

[2] www.vetinst.lakselus and www.imr.lakselus

more sea lice and more treatments or other efforts to control sea lice infestations (Jansen et al., 2012). In Norway, the surveillance of salmon lice on farmed salmonids was the responsibility of the Animal Health Authority (AHA) until 2004, when AHA was replaced by the Norwegian Food Safety Authority (NFSA). A national action plan was implemented by AHA in 1997, as a consensus tool between authorities, fish farmers and fish health personnel (Eithun, 2000). This plan set legal limits for the maximum amount of salmon lice allowed on the farmed salmon and protocols for compulsory reporting, strategic regional treatments and monitoring of salmon lice infections in wild salmonids. In a review of the action plan (Heuch et al., 2005) it was argued that with the current (2003) volume of salmon farming and level of lice control, it would be unrealistic to expect no negative effects on wild salmonid populations. Lower maximum limits for salmon lice in farms and more detailed electronic reporting of raw lice data from the farms were also suggested. The measures used to count, report and treat against salmon lice at fish farms today (2018) are defined in the salmon lice regulation (Norwegian Ministry of Trade, Industry and Fisheries, 2012). The maximum limits are 0.2 female salmon lice during spring and 0.5 females during the rest of the year. The protocol for counting salmon lice at the farms has changed over the years, and reliable and comparable data on abundance are available only from 2012 onwards (Helgesen, pers. com.). In addition, the regulation describes how farms must implement an integrated pest management strategy, with coordinated zones for production and fallowing and with coordination of treatment methods and timing between the companies within the production zones.

Figure 5. The author looking for sea lice on salmon farmed in closed cages, October 2017, supervised by a TV-crew from Pandora Film AS (Photo: Asle Haukås, Norwegian Veterinary Institute).

Figure 6. Sea lice on salmon in the reference net-pens at site 1 in 2013 (Paper I). A variety of L. salmonis and C. elongatus were present on most of the fish in all the net-pens at the project sites. The tail fin and pelvic fins were often lacerated after repeated treatments (Photo: Arve Nilsen).

Drug resistance

Drug resistance in parasite populations can be defined as a 'genetically-based decrease in susceptibility to a pesticide' (Tabashnik et al., 2014), with profound (and negative) implications for the possibility of regulating parasite populations through chemical interventions. Contemporary knowledge about drug resistance in salmon lice is described thoroughly in three doctoral thesis (Fallang, 2005; Helgesen, 2015; Aaen, 2016). Decades of treatments with pesticides (Aaen et al., 2015) induced resistance against organophosphates (Denholm et al., 2002; Fallang, 2005), pyrethroids (Sevatdal and Horsberg; 2003, Helgesen et al., 2014), emamectine benzoate (Jones et al., 2013) and hydrogen peroxide (Treasurer et al., 2000). Increasing drug resistance has been the cause of increased drug use in all salmon farming countries in the period after 2000 (Denholm et al., 2002; Helgesen and Marin, 2018), and has been described as an increasing problem in Norway since surveillance started in 2013 (Grøntvedt et al., 2014; Helgesen et al., 2019). With more pharmaceuticals poured into the cages and released into the marine environment, there has also been growing concern about the risk of harming non-target species (Samuelsen and Agnalt, 2018; Urbina et al., 2019). Research on the environmental impact of sea lice treatments is not yet conclusive. However, a steadily growing awareness about the possible impact of such releases of drugs into the surrounding water bodies points towards a need for more caution. The Norwegian authorities consequently had to regulate where well boats release water containing drug residuals (pyrethroids and hydrogen peroxide) in order to protect local breeding areas of shrimps and marine fish from potential harmful effects (Norwegian Ministry of Trade, Industry and Fisheries, 2019).

The rotation of drugs and the elimination of parasites through other, non-medicinal measures will reduce the selective pressure towards resistance. However, once an inheritable resistance mechanism has developed and has spread in a population of parasites this mechanism will most likely stay present in the population for a long period (Helgesen and Marin, 2018). Sensitivity could increase again if mitigating practices such as restricted use of a specific drug and with application of other therapeutic measures (Helgesen, pers.med). If a specific drug is reintroduced, the mutations coding for drug resistance will most probably multiplicate swiftly and effectively. In other words, using a metaphor from the Norwegian marine food industry;

there is no way to get the caviar back into the tube. It is fair to state that the age of drug control of salmon lice in Norway was coming to an end by 2012. The use of drugs increased from 2009, as a compensation for gradually reduced effect of both bath treatments and in-feed medication (Helgesen and Marin, 2018), and there was no growth in Norwegian salmon production in the years after 2012. New brooms had to be found and used, but could they sweep away lice as efficiently as the drugs used to do, or would they just whirl up more dust?

The cleaner fish fallacy

One of the most commercially successful strategies against drug-resistant salmon lice has been revitalising the use of cleaner fish. Several fish species have developed a specialised behaviour whereby they pick and eat external parasites from the skin of other fish. In salmon farms, wild fish of the labridae species were traditionally used for this purpose, e.g. the goldsinny wrasse (*Ctenolabrus rupestris*) and ballan wrasse (*Labrus bergylta*) (Bjordal, 1991). Other labridae species have also been used, although the delousing effect of these is less certain (Nilsen et al., 2014). Trials with farmed ballan wrasse started in Norway in 2012 (Skiftesvik et al., 2013, Leclercq et al., 2014). After successful trials with the production of lumpfish (*Cyclopterus lumpus*), this has now turned into the second largest aquaculture production species in Norway. In the period 2012-2017, the number of cleaner fish increased from 13.9 to 54.6 million, where 29.7 millions were lumpfish (Norwegian Directorate of Fisheries, 2018b).

Given today's massive use of cleaner fish, it is possible to argue that salmon producers have attempted to wipe out one problem (salmon lice) by creating at least four others: (1) depleting wild wrasse populations, (2) genetic disturbance of local cleaner fish populations, (3) high cleaner fish mortalities and (4) the possibility of cleaner fish as vectors for diseases transferrable to salmon or to other cleaner fish outside the cages (Treasurer, 2012; Karlsbakk et al., 2013; Munro et al., 2015; Gulla and Bornø, 2018; Powell et al., 2018). Despite the ingenuity displayed through rapid development of equipment and procedures for more effective use of cleaner fish, it is to my opinion difficult to see this as a sustainable way to combat salmon lice in commercial salmon farming.

Non-medicinal treatment methods

The main driver behind the increase in new treatment methods has been the upholding of strict salmon lice regulations while the previously effective 'miracle drugs' have gradually lost their therapeutic effect, as discussed above. Recent studies of the immune response during attachment of salmon lice copepodites have boosted the optimism towards development of effective vaccines in the foreseeable future (Evensen, pers. com). However, together with selective breeding towards salmon lice resistance, vaccination is still more of a possible solution for the future. As drug-resistant salmon lice became widespread, the number of treatments and the methods used to control salmon lice in Norwegian fish farms changed dramatically (Helgesen and Marin, 2018; Overton et al., 2018). A broad variety of new treatment practices is described, ranging from the relatively simple procedure of bathing the salmon in fresh water to complex machinery developed to pump and move the fish between cages while exposing them to high pressurised water or temperate (warm) water. In April 2010, I participated in the monitoring of the first prototype of mechanical delousing equipment (Nilsen et al., 2010). The first mechanical treatments in the national database for treatments (Folkehelseinstituttet) were recorded in 2011. By 2014, 177 mechanical treatments were recorded, representing less than 5% of the total 3,654 prescriptions that year. Bath treatment with fresh water has been used with some success as a treatment against amoebic gill disease (AGD) in Tasmania since the mid-1980s (Powell et al., 2015) and in Norway since 2012 (Hytterød et al., 2017) while application for use against salmon lice on a commercial scale is relatively new (Stone et al., 2002). For a brief period in 2016, most lice treatments were performed with different bath treatment protocols (e.g. fresh water or combinations of different drugs), but in 2017 the majority of delousing operations were based on the two existing thermal delousers commercially available; Thermolicer® (Grøntvedt et al., 2015) and Optilicer® (Roth, 2016). Other methods for removing sea lice include the use of laser technology[3] to identify shoot and kill the sea lice on the salmon; but to my knowledge, this has until now been performed without any thorough scientific documentation of the effect.

[3] www.stingray.no

A review of treatments against salmon lice in Norway (Overton et al., 2018) calculated a 40% increase of delousing operations from 2012 to 2017, all treatment methods included. These figures could be somewhat exaggerated, because traditional medical treatments are typically conducted at all cages at the sea site, while non-medicinal treatments are more often applied at cage level. Mechanical and thermal treatments are also less efficient against the attached chalimii than pyrethorids and emamectin benzoate used to be, and this could partially explain the need for more frequent treatments in the last few years. Besides increased use of cleaner fish, other measures, like surrounding the cages with skirts, were also implemented to slow down salmon lice reproduction and spread. The Norwegian Seafood Association has claimed that Norwegian salmon farmers have executed an environmentally responsible strategy by abandoning medical treatments (Kvistad, 2018). It seems more reasonable to say that drugs went out of business because of widespread salmon lice resistance and that the development in the period from 2012 to 2017 could be described as an '*industry dealing with an escalating problem*' (Overton et al., 2018).

1.5 Fish welfare

In this thesis, I focus on the welfare of farmed Atlantic salmon (*Salmo salar*), with a few examples and references from studies of other species, mostly other salmonids. Atlantic salmon have been the dominant species in aquaculture production in Norway since 1978 (Norwegian Directorate of Fisheries, 2018a), and salmon has always been a species of particular interest. The first Norwegian legislative protection of salmon is dated to the text 'Gulatingsloven' with an origin of around 1000 AD: '*The gift from God* (i.e. the migrating salmon) *must be allowed to travel from the mountains to the ocean*' (Robberstad, 1937). This protection of the migrating salmon was probably most of all a protection of the proprietor rights of the landowners along the rivers, and it has been a long journey towards the present legislation and debate about fish welfare.

What we talk about when we talk about fish welfare

Most reviews of research on fish welfare support the view that fish are capable of a conscious emotional response to nociception or danger. A thorough review of fish-centred moral philosophy described the knowledge about fish physiology, suggested methods for evaluating welfare and described how human activities affect fish welfare (Huntingford et al., 2006). They called for a better understanding of fish mental capacities and argued that fish exhibit behavioural needs on the basis that we have sufficient evidence to support the theory that fish are sentient beings and that we need to develop more precise and useful welfare indicators. When looking at the philosophical and scientific theories about fish in our Western societies, sentience is commonly considered an important determinant, and one of the most important criteria for the inclusion of animals in our moral circle (Lund et al., 2007). Although good health, and thus good productive capacity, is essential to welfare, good health does not necessarily mean good welfare (Ashley, 2007). Animal ethics based on a combination of the animal's interests, needs and inherent nature could be a possible bridge between the often individualistic and animal rights-centred ethics of leading philosophers such as Tom Regan and Peter Singer and the empirical realm of animal welfare scientists (Fraser, 1999).

After a long period dominated by so-called positivism[4], there has been a renewed interest in the existence and importance of animal emotions (Fraser, 2009). In recent animal welfare literature it is common to refer to three different objectives for improving animal welfare: (1) to ensure good physical health and functioning of animals, (2) to minimise unpleasant 'affective states' (pain, fear, etc.) and to allow animals normal pleasures, and (3) to allow animals to develop and live in ways that are natural for the species (Fraser, 1999; Fraser, 2003; Fraser, 2009). These objectives lead to three different approaches used to assess animal welfare, often defined as function-based, affective state-based (or feelings-based) and nature-based. In this thesis, I will use the three approaches described by Fraser as the framework for discussing the quality of fish welfare described in the four presented papers.

[4] https://research-methodology.net/research-philosophy/positivism

To assess welfare in a scientific way, we need measurable welfare indicators (Broom, 1986; Dawkins, 2004; Martins et al., 2011; Noble et al., 2018). We can use welfare indicators describing the environment and management (resource-based), the fish (animal-based) or both. Common resource-based indicators are dissolved oxygen (DO), carbon dioxide (mg/L), water temperature (°C), stocking density (kg/m^3) and water velocity (cm/s). These indicators are relatively objective, possible to measure with standardised equipment and methods, and easy to use in statistical analysis and presentations. Examples of animal-based indicators are prevalence of external lesions such as fin lesions, ulcers and cataracts, pathological changes in internal organs, fluctuations in blood chemistry or plasma hormones and observations of fish behaviour. Many of these indicators are also quantifiable through scoring systems; others depend on the observer's skills or the test procedure; and for some there is not sufficient data to be able to distinguish between 'normal' or 'abnormal' observations. However, the information we extract from examining the fish is vital to assess how the fish respond to their environment. The British biologist and ethologist Marian Stamp Dawkins (2004) has argued for the use of behaviour as the most important welfare indicator: *'All of the measures that we might want to use have to be validated in terms of the extent and effectiveness with which they tell us about animal health and about what the animals themselves want'* (My underlining).

As a welfare indicator, behaviour is difficult to interpret. Fish with severe lesions, such as ulcers or during recovery after various types of surgeries, may keep swimming almost as normal despite a possible experience of discomfort or pain (Rose et al., 2014). Descriptions of species-dependent behaviour (Martins et al., 2011a) and the discovery of the individual coping styles of fish (Koolhaas et al., 1999, Martins et al., 2011b) add to the complexity of using behaviour to assess the physiological and mental state of fish. From my personal experience, personnel at fish farms often use behavioural welfare indicators in one way or another. They use swimming depth, schooling and feeding behaviour to evaluate health status and interpret erratic swimming or lethargy as signs of disease or weakness in individual fish. In a review of behavioural indicators of welfare in farmed fish (Martins et al., 2011a), it was argued that the rapid development of cameras and image processing would soon lead to more sophisticated behavioural monitoring in fish farms. Individual recognition may even become possible. However, our use of and understanding of fish behaviour to assess fish welfare in a systematic and

documentable way in commercial aquaculture systems is still meagre (Noble et al., 2018).

Returning to the three approaches used to assess animal welfare, from a function-based point of view, assessment will include a thorough evaluation of animal-based data, e.g. health, stress indicators, fin lesions, skin lesions, growth, condition factor and mortality. A function-based approach would also lean on the most vital resource-based data such as DO and temperature to evaluate the environmental impact on the observed biological data. The strengths of the functional approach are the relative objectivity of the measurements and the strong link to commercial relevance; furthermore, this approach provides answers to the first of Marian Dawkin's basic welfare questions: 'Are they healthy?' (Dawkins, 2004). However, if sentience is the key consideration, then the main aim should be to remove or reduce suffering and allow the animal to feel well. This leads us to the affective state approach (Huntingford et al., 2006). In this context, observations of animal-based indicators of subjective feelings are important, e.g. pain, fear or distress. Resource-based indicators like density/available area or volume, and the presence of potentially damaging equipment or procedures are also important, because the possibility to exercise or play and freedom from fear or physical injuries is necessary to uphold a positive affective state. The strength of this method is the possibility to find answers to the second of Dawkin's questions: *'Do they get what they want?'* (Dawkins, 2004) and the possibility to adjust the environment and husbandry practices to prevent suffering. An obvious weakness is the methodological problems connected to standardising protocols and interpretations, i.e. how to make the inference from welfare indicator scores to the subjective state of the animals. The third approach is the nature-based or fish preference approach: '*welfare is the internal state of a fish when it remains under conditions that were freely chosen*' (Volpato et al., 2007). The nature-based approach takes into account the physiological and behavioural requirements of the animals and asks to what extent animals can choose and fulfil their species-specific and individual needs. This approach builds on the basic assumption that animal welfare will be maximised by exposure to an environment as close to the natural habitat as possible and with access to display natural, species-dependent behaviour. Again, a combination of both animal- and resource-based indicators is necessary. Animal-based indicators focus on scoring the fulfilment of a defined set of natural behavioural patterns and

probably also the absence of aberrant behaviour such as stereotypic movements or excessive fearfulness. It would further be necessary to measure whether the resource-based environmental indicators correspond to conditions regarded as natural. One strength of this model is its obvious appeal to human moral considerations (what could be better than 'natural'?), and nature-based models are often considered important in organic animal husbandry systems. This model also urges a deeper understanding of the complex biological and psychological needs of different animal species. The most obvious shortcoming is the fact that farm animals are not only a product of natural selection, as with their wild counterparts, but are also largely a result of the human-driven selective breeding system often referred to as domestication. Behaviour that was positive in a natural habitat (e.g. fear of predators) can lose its protective quality in a captive environment. Instinctive behaviour (e.g. seasonal overfeeding to survive periods of hunger) can be detrimental in domestic animals provided with free access to high-energy diets. Unnatural and stressful or painful treatments like vaccination or medical treatments can be necessary to avoid infections or death (Fraser, 1999). For domesticated animals (including *Homo sapiens*), nature is no longer necessarily a natural place to be.

Fish welfare – who cares?

In our modern world, the food security of billions of people is reliant upon industrialised animal husbandry. This is a situation where the question of animal welfare is not only a matter of philosophical and ethical considerations; the answers to this question will have profound political, economic and practical implications. In Western culture, there is general agreement that at least some animals are entitled to certain moral respect and animal welfare considerations (Lund et al., 2007). When we give creatures (species or groups or individuals) access to our moral circle, we also have to give their interests serious moral consideration for their own sake (Singer, 1981). It is reasonable to argue that humankind's moral horizon or 'moral circle' has widened gradually over the millennia. However, since World War II, we have industrialized our husbandry systems and now we breed and stock farm animals in prison-like premises for the benefit of human consumption of relatively low-priced animal protein. As I see it, this is possible only because the lives and well-being of farm animals are at least partially outside our

moral consideration. We accept some individual animals as a part of our group, especially pets or companion animals. We could also grant other animals a moral consideration in their own right, like whales and great apes. The same is the case with the many injured wild animals that are nursed back to recovery and later released back into their habitat. People tend to care more about baby seals and other cute animals as opposed to pest animals (such as rats) although they are believed to possess the same capacity to suffer. This could also be explained by a psychological mechanism evolved over thousands of years where humans are adapted to connect to and interact with individual animals as part of our group, but have less capacity to activate empathy or moral responsibility towards larger groups of both wild and domesticated animals (Børresen, 1994).

The question of whether we should give animals, cute and ugly alike, general access to our moral community has been discussed for at least 2,000 years, with strong arguments for and against. The Greek philosopher Aristotle (384-322 BC) stated, *'Equal should be treated equally, and unequal unequally'* (Barnes, 1984), and in Western culture animals were not judged to have a moral status because they, according to leading philosophers and representatives from the Church, lacked the ability to speak and reason, and were thus regarded as soulless machines (Christoffersen, 2000). The major change in our view on animals came with Jeremy Bentham (1748-1832) and his famous quote: *'The question is not, Can they reason? nor, Can they talk? but, Can they suffer?'* (Bentham, 1789). Without diving further into the philosophical depths of welfare theory, we will keep our focus on aquaculture and salmon farming. How should we deal with fish welfare in modern salmon farming in practice?

Animal welfare is a priority area for the World Organization for Animal Health (OIE) (Anonymous, 2018a) and for the European Food Safety Authority (EFSA) (Håstein, 2005). In Norway, fish has explicitly been included in the Animal Protection Acts since 1974, and the protection of fish welfare was strengthened in the Animal Welfare Act of 2009 (Norwegian Ministry of Trade, Industry and Fisheries, 2009). If we expand our moral circle to include fish, for whatever scientific or philosophical reasons, we must also define what welfare means in practical fish farming (Conte, 2004; Huntingford et al., 2006; Ashley, 2007; Lund et al., 2007). For my thesis, I have been working with salmon production and welfare in a new farming technology. My objective was to

investigate water quality and rearing conditions in CCS and evaluate their impact on fish performance, including health and welfare. Therefore, I mostly ended up with a function-based welfare approach. Nevertheless, the documentation of health and diseases can also be considered important for a feeling-based welfare approach (you need to be healthy to feel well). Preventing extreme temperatures and securing a preferred water quality and water velocity could be seen as both affective state and nature-based welfare measures. If the fish are not provided with a physical environment within their range of natural preferences, it is hard to argue that they are being given the benefit of choosing. And with environmental conditions outside the physiological window of tolerance, the fish could be at risk of being disturbed or distressed, even before a specific negative impact on the biological output could be identified. However, for the salmon swimming inside the tanks or the cages, any theoretical and philosophical distinctions are probably not that important.

In the 2017 annual report from NVI (Hjeltnes et al., 2018), it is emphasised that all aspects of welfare are relevant, but in commercial farming they recommend focusing especially on functional- and feelings-based welfare indicators. In the annual reports from both NVI and IMR, function-based criteria and feelings-based criteria are used to evaluate fish welfare in salmon farming. Mortality, as an example, is a relevant, but crude functional-based welfare indicator. So far, fish farms are obliged to report salmon lice counts to the authorities (Norwegian Ministry of Trade, Industry and Fisheries, 2008) together with lice treatments, water temperature, number and weight of fish and total feed consumption. Cleaner fish mortalities are not reported, despite reports describing potentially high mortalities in all cleaner fish species (Nilsen et al., 2014; Hjeltnes et al., 2018). Mortality of salmon (and rainbow trout) is the welfare indicator described and discussed in the annual risk assessment form the Institute of Marine Research (IMR) (Stien et al., 2018a). In the latest annual reports from NVI (Hjeltnes et al., 2018), fish welfare during delousing using new non-medicinal technologies has been the main topic. They used a function-based approach and evaluated animal-based welfare indicators such as prevalence and severity of skin lesions and mortality rates. With regard to the use of temperate water to remove salmon lice, there has also been a heated discussion about how the salmon perceive the sudden increase in water temperature up towards 34 °C. This practice has been criticised because salmon, like rainbow trout,

might have nociceptors that are activated by the water temperatures used in the thermal delousings (Ashley et al., 2006). Recent studies (Gismervik, pers. com.) has shown signs of pain in salmon exposed to warm water and NFSA is now (April 2019) warning the industry and fish health personnel against delousing with higher temperatures than 28 °C. This clearly shows how important the mental state of the fish has become in the present execution of animal welfare regulations in Norway. The outcome of this development in terms of sea lice management at the sea sites throughout 2019 is uncertain.

Taking into consideration the vast profits generated by salmon production, even if there was some doubt about fish sentience, we should be morally obliged to put a lot more effort into acquiring and using knowledge to improve all aspects of fish welfare in salmon farming. In many cases, it would make sense to design fish welfare studies which use both resource-based and animal-based indicators and interpret these data using biological functioning-, affective state- and nature-based approaches. This methodology has been implemented in a suggested model for the overall assessment of the welfare (SWIM 1.0 and SWIM 2.0) of caged Atlantic salmon (Stien et al., 2013; Pettersen et al., 2014). In the studies described in this thesis, we have used resource- and animal-based indicators and have interpreted this data mainly from a biological functioning point of view, but without the hierarchic modelling described in the SWIM-models. However, where possible, we have also recognised the validity of the other approaches to the concept of animal welfare and in this thesis I have interpreted our data in the context of the emotional and/or behavioural preferences of farmed salmon.

1.6 Closing in on closed cages

Two main drivers behind the recent years with development of alternative farming technologies have been the increasing problems with salmon lice and increased focus on the possible genetic introgression from escaped farmed salmon. Industrialized farming of Atlantic salmon and rainbow trout in seawater has been developed for marine net-pens with a free exchange of water, pathogens, parasites and organic effluents with the marine ecosystem outside the cages. In this context, aquaculture production depends on available local ecosystem services (FAO, 2007), e.g. areas for location of sites, clean water and a large recipient capacity. Alternatives to traditional

net-pens are (1) floating, closed confinement systems (CCS) with rigid walls or with flexible walls, (2) offshore constructions, (3) land-based flow-through systems and (4) land-based recirculating aquaculture systems (RAS). The Norwegian management system of aquaculture is complex, involving County authorities, the Norwegian Directorate of Fisheries and the Ministry of Trade, Fisheries and Aquaculture. Between 2000 and 2015, the Norwegian government issued new licenses for salmon farming through six different allocating rounds (Hersoug et al., 2019). From 2002 to 2009, 155 new licenses were issued, with no environmental strings attached. In the licensing round in 2013, 45 green and so-called 'super-green' licenses were issued. The idea was to incite development of new farming technologies and to reduce the environmental impact caused by traditional, net-pen farming (Hersoug et al., 2019). Annual reports from some of these projects are published at the Directorate website[5]. As the pressure towards access to more licenses increased, the government issued a new allocation round already in 2015, referred to as 'Development licenses', again with environmental issues as the most important drivers. This round was, unlike the previous, not restricted to a specific number of licenses, but encouraged new and ambitious projects to apply for the number of licensed they could envisage necessary to implement the new technology. 'Development licenses' were also supposed to support in resolving the environmental and area-related challenges addressed by the new 'traffic-light system' (Hersoug et al., 2019). Little information has been aggregated about the performance of the implementation of 'Development licenses' so far, besides the project brochures presented at the Directorate's website[6]. A controversial aspect of these licenses is how such licenses (as with previous rounds) can be converted to standard, commercial licenses after finishing a specified technical and biological test program (Hersoug et al., 2019). The price for converting 'Development licenses' is NOK 10 million, less than 10% of their assumed present market value. The possibility of harvesting such profit margins through converting licenses could be possible driver behind some of the largest and most spectacular technological projects. The large offshore projects have been suggested to increase the available area for fish farming (Anonymous, 2018b), but it is

[5] https://www. fiskeridir.no/Akvakultur/Delt-kunnskap-og-erfaring/Groene-loeyve
[6] https://www.fiskeridir.no/Akvakultur/Tildeling-og-tillatelser/Saertillatelser/Utviklingstillatelser/Kunnskap-fra-utviklingsprosjektene

also a risk with large offshore farming projects that problems of lice and emissions are moved to new and until now pristine areas outside the coastline.

The CCS described in this thesis belonged to group (1): floating CCS with flexible walls. These CCS were floating tarpaulin bags (Figure 7), with single flow-through of seawater, and with oxygen supplied through diffusors or ejector systems. So far, there has been no aeration or removal of CO_2 in these systems. In the outlet, particles were separated, but the remaining outlet water was left untreated. The inlet water was pumped from a 25 m depth and neither filtered nor disinfected to remove viruses, bacteria or parasites. Less sea lice and microbial pathogens could be an advantage when using water from such depths. However, for other parasites or for potentially troublesome pathogens or opportunistic marine pathogens such as *Moritella viscosa* or *Aliivibrio* spp. the risk might even increase. Filtration and disinfection of intake water has been suggested as a measure to improve the biosecurity of closed containment systems (Rosten, 2011, Espmark, 2019).

Social and environmental impact of CCS technology

Salmon lice, release of chemicals during lice treatments, escaped fish, organic emissions, energy use, emissions of CO_2, and the environmental impact of feed production for farmed fish are examples of negative externalities, on both a local and a global scale. Life cycle assessment (LCA) (Finnveden et al., 2009) is a tool widely used to assess the sum of environmental impacts and resources used throughout a product's life cycle. In an LCA of Canadian aquaculture systems, including CCS cages, land-based systems had the poorest environmental performance, mainly because of the energy needed to pump and treat water (Ayer and Tyedmers, 2009). In the case of the marine cages, CCS cages had a smaller environmental footprint compared to net-pens, if given access to low-CO_2 energy sources like hydroelectricity. If the extra energy needed to run CCS was supplied via fossil fuel-based electricity, the balance would shift in favour of net-pens. However, this analysis was executed without considering the environmental impact of sea lice and sea lice treatments and without the technological possibility of collecting organic emissions from CCS or land-based flow-through systems.

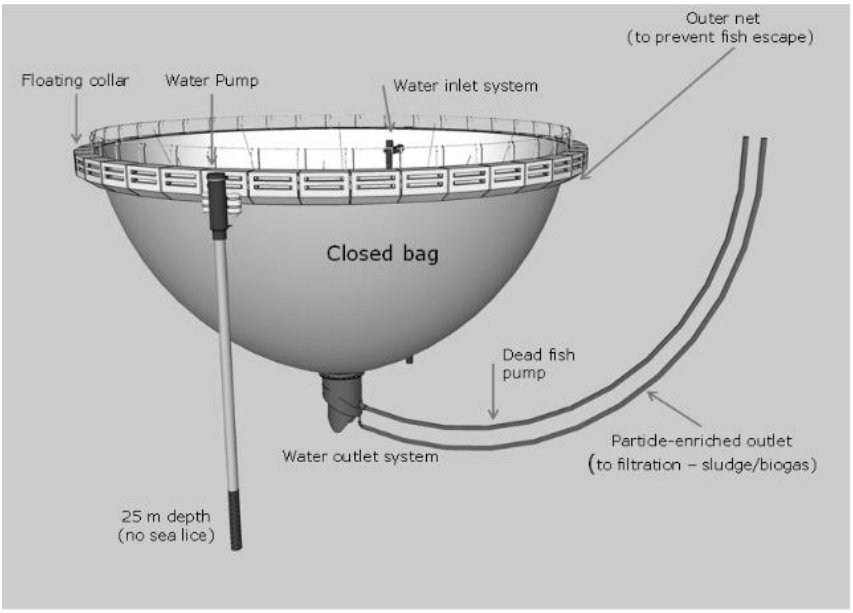

Figure 7. Above: Schematic illustration of a floating, tarpaulin cage (2870 m³) with water intake from a 25 m depth, described in Papers I, II, III (Illustration: AkvaDesign AS). Belowt: Picture of a site with ten floating, tarpaulin cages (6000 m³) of the same basic design, used for trials described in Paper II (Photo: AkvaFuture AS and Visual 360).

Thus, the outcome of their analysis is of limited value for evaluating such systems in a Norwegian context. In a comparison of the carbon footprint of marine net-pens (Norway) and land-based freshwater RAS (US) (Liu et al., 2016) concluded that freshwater RAS located in the US released twice as much CO_2 during the production period as salmon production in Norwegian net-pens. After adjusting for the airfreight needed to transport Norwegian salmon to the US, the balance would be reversed, with RAS releasing less than 50% of the CO_2 emitted by Norwegian net-pens. This is an interesting twist to the discussion about the ecological sustainability of salmon production in clean Norwegian coastal areas. It also highlights how land-based RAS is a technology that may have greater global potential than cage-based farming systems. Salmon farming based on marine cage systems is today mainly located in Northern Europe, Canada, Chile and Oceania. With more effective land-based technology available, this picture could change. However, poor system designs, water quality issues and mechanical problems have so far been important constraints on the development of commercial-scale RAS production worldwide (Badiola et al., 2012).

Outside the scope of this thesis, there are several important environmental issues that should be included in assessments of the sustainability of intensified salmon farming. Several research projects are now initiated to explore the possibility of using CCS salmon farming technology to develop more diversified aquaculture systems, so-called Multitrophic Aquaculture (MTA) (Stedt, 2018). CCS farms close to mainland infrastructure could exploit more environment-friendly energy sources (Ayer and Tyedmers, 2009). Collection and reuse of faeces and surplus feed could contribute to reduce the environmental footprint of industrialized fish farming in vulnerable coastal areas. An increased demand for protein-rich and high-energy feed for carnivore salmonids could increase the competition for important feed inputs like captured fish and vegetable oils. Emission of greenhouse gases is also a challenge, through energy-consuming production systems and through trans-continental transport of both feed ingredients and the salmon products. The salmon farming industry in Norway often present increased volumes of salmon production as a means of improving the global supply of fish for human consumption (Anonymous, 2018c). However, it could be argued that a further expansion of the aquaculture industry without a shared vision between public and private sectors on how to develop fish farming with less negative external

costs could be a threat, not only to the surrounding environment, but also to itself (Naylor et al., 2000).

Closed containment systems: Where are we and where are we going?

In the period from 2012-2015, when the projects leading to my thesis were developed, very few studies had been published on the actual production capacity and the possibilities and pitfalls of fish welfare in commercial-scale closed cages. As I finish this thesis (April 2019), knowledge about the management and biological performance of large, closed containment systems is still scarce. A thorough review of the literature on the biological requirements for post-smolt Atlantic salmon in closed containment systems was published in 2011 (Thorarensen and Farrell, 2011), together with a Norwegian report on the possibilities of CCS technology (Rosten, 2011). Since 2011, several CCS projects have been implemented. The most profiled projects with activity in the years from 2011 to 2019 are:

1. The Neptun cage, 21,000 m^3 solid wall CCS, Mowi AS
2. Preline, 2,000 m^3 raceway CCS, Lerøy AS
3. Aquadomen, 5650 m^3, solid wall CCS, Cermaq AS
4. Flexible and solid wall CCS, different volumes, Nekton AS
5. Flexible wall CCS, 2870 and 6000 m^3 volumes, AkvaDesign AS

Projects 1, 2, 3, 4 are incorporated in the CtrlAqua research consortium, headed by NOFIMA (Espmark, 2019), while the projects developed by AkvaDesign AS and AkvaFuture AS that are described in this thesis are not. Between 2011 and 2014, few new studies were published on CCS-related topics, but from 2015 onwards, more information became available from experimental studies and from the first field trials involving CCS technology projects (nos. 1-3 from the list above). Two doctoral theses (Calabrese, 2017; Sveen, 2018) have been published, together with a few papers with reference to CCS-technology: hydrodynamic studies (Gorle et al., 2018; Klebert et al., 2018; Maximiano et al., 2018; Gorle et al., 2019) and a survey of technical specifications of large land-based tanks and one pilot CCS (Summerfelt et al., 2016). Growth, mortality, muscle development and cardiac development in net-pens and a raceway CCS has been compared (Balseiro et al., 2018). The microbiota in recirculating aquaculture systems

(RAS) and the 21,000 m^3 Neptun cage is described (Rud et al., 2017), and development of the skin barrier of Atlantic salmon after sea transfer to the raceway CCS (Karlsen et al., 2018). Several experimental studies of stocking density and specific water consumption have been published, with special reference to implementation in CCS or in RAS systems (Sveen et al., 2016; Calabrese et al., 2017; Sveen et al., 2019). The effect of swimming exercise in CCS model cages is described in this thesis (Paper IV) and exercise in combination with salinity by Hvas et al. (2018) and the effect of different temperatures on swimming capacity of salmon by Hvas et al. (2017a). The impact of intensification on levels of CO_2 in large-scale CCS cages from AkvaDesign AS is described in this thesis (Paper III), and the effects of CO_2 in RAS in two recent publications (Good et al., 2018; Mota et al., 2019). The effect of CO_2 on post-smolt Atlantic salmon has been described (Fivelstad, 2013; Fivelstad et al., 2003; Fivelstad et al., 2015; Fivelstad et al., 2018), it has been shown a diurnal variation of CO_2 and TAN excretion of post-smolt at different water flow (Kvamme et al., 2019). The effect on sea lice infestations is described in this thesis (Paper I). A few master theses describe different aspects of CCS-based farming (Chen, 2015; Pedersen, 2016; Haaland, 2017; Stedt, 2018). A large number of important scientific studies with relevance to CCS technology were conducted in the late 1980's and early 1990's describing the ongrowing of post-smolt salmon in land-based, flow-through tanks supplied with oxygen-enriched seawater. These studies are still very relevant and important for the understanding of closed confinement farming (Kjartansson et al, 1988; Fivelstad et al., 1990; Fivelstad et al., 1991; Fivelstad and Smith, 1991; Forsberg, 1994; Fivelstad et al., 1995; Forsberg 1995a,b; Forsberg, 1996; Forsberg and Bergheim, 1996; Sanni and Forsberg, 1996; Forsberg, 1997; Fivelstad et al., 1999). In addition, an early pilot study on the on-growing of post-smolt salmon in closed, tarpaulin covered cages (CCS) was performed in Southwestern Norway (Skaar and Bodvin, 1993).

Studies on stocking density, specific water consumption and salinity and exercise from experimental studies with post-smolt were described in a doctoral thesis (Calabrese, 2017). One of the conclusions was: '*Large scale CCS studies are needed to verify results in this thesis*' (p. 34). This thesis is an attempt to provide more detailed insights about rearing conditions, production capacity and fish welfare in such systems.

2. Knowledge gaps

At the time when this study was initiated (2014), several new technology projects were under construction, but with no published data on the production of Atlantic salmon in large-scale closed containment systems. There were several assumptions about the possible benefits and problems connected to the production of salmon in CCS: prevention of salmon lice and possibly other infectious diseases, better management of water velocity and water temperatures, the challenge of safeguarding water quality, and also the risk of acquiring new disease problems. According to the Norwegian Aquaculture regulation §20, acceptable standards of fish welfare must be documented before any novel methods, installations or equipment can be used in commercial fish farming. However, no detailed instructions were given about how to interpret and implement this regulation.

- There was a need for thorough investigations of fish welfare in new cage technology projects. Furthermore, it was necessary to perform studies on how to assess fish welfare during commercial CCS salmon farming.
- Close monitoring of a CCS project should be carried out over several production periods with the aim of (1) generating information about technological constraints and possibilities and to (2) point out the most important fish welfare issues.

3. Aims and objectives

The main aim of this thesis was to evaluate production of Atlantic salmon (*Salmo salar*) in a new closed confinement system (CCS), with focus on prevention against salmon lice, quality of rearing conditions, growth, mortality and fish welfare.

Objectives:

1. Describe the abundance and infestation dynamics of sea lice (*Lepeophtheirus salmonis* and *Caligus elongatus*) on Atlantic salmon in CCS and compare with the results from net-pens.
2. Describe growth rates, mortality rates and mortality causes during production of Atlantic salmon in CCS and compare with data from net-pen production and, if possible, other CCS projects. Production data should also be combined with studies of water quality and other welfare indicators to interpret the production capacity in a broader fish welfare context.
3. Establish models for maximum production capacity and describe how intensified production in CCS affects water quality and fish welfare.
4. Investigate the effect of water velocity and temperature on growth and welfare through an experimental study with small test cages (40 m^3).

4. Methodological considerations

This thesis is a mixture of different study designs and methodological approaches. It is most of all a descriptive study monitoring results from one large, commercial-scale closed confinement system (CCS), also referred to as closed cages. The main input from these test cages is farm data, e.g., temperatures, oxygen levels, number and size of fish, sea lice counts, water flow rates, feed use, mortalities and weight controls. We collected data from three different companies, at five sea sites, over a period of five years. It is not possible to give an exhaustive account of all the possible pitfalls in such a process, but I will briefly go through the factors I believe were the most important sources of data error and describe how we dealt with this along the way. I also give a brief description of how the study design was developed and discuss some of the ethical considerations.

How the project was developed

First, it will be useful to give a short description of the study designs, the sites and how we developed the project from May 2012 to May 2017. It all started with a pilot CCS (1500 m^3) at site 1 in May 2012. AkvaDesign AS, a small company located in Brønnøysund, developed and patented the technology [7]. The first pilot study showed 100% protection against salmon lice. Challenges with suboptimal water quality highlighted the immediate need for technical improvement. After finishing CCS no. 1 (1550 m^3), new smolt were stocked in the next CCS with double size (2870 m^3). At site 1, we were allowed to use both CCS and net-pens, facilitating a cohort design during the trials from May 2012 to January 2015. This was favourable for the sea lice research; at the same time, we obtained comparable data on mortality and growth rates from both systems (Paper I). Cage no. 13 in Paper I was stocked with post-smolt from a net-pen at site 3, allowing us to count sea lice also in a closed cage with a parallel group stocked in a net-pen ringside. The lice counts from CCS where the salmon had been exposed to moderate salmon lice infestations showed gradually reduced salmon lice abundance, and this effect is an important part of the discussion in Paper I.

[7] search: 'AkvaDesign' at https:// search.patentstyret.no

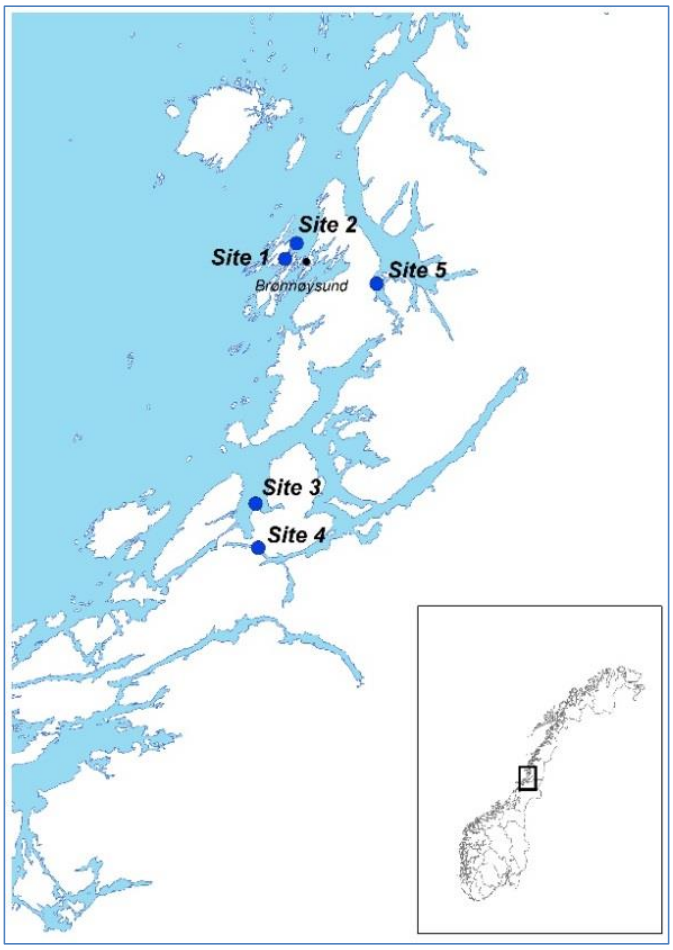

Figure 8. The locations of the five sea sites used during the trials (Illustration: A.Tarpai).

In 2013, The Ministry of Fisheries and Coastal Affairs issued 45 'green licenses', allocated to new farming technologies. Two of those licenses were granted to the two commercial companies already cooperating with AkvaDesign AS, based on the use of AkvaDesign's closed cages. A new research site (site 4) was located in Bindalsfjorden, Nordland County, close to the hatchery that supplied smolt to the project. At site 4, production of post-smolt <1 kg in four CCS (2870 m^3) was conducted as part of the two 'green licenses'. With sea transfer of S0 smolt in October to November, the farm had the benefit of obtaining warmer and salmon lice-free water from a 25 m depth during winter before transferring the fish to net-pens in April to May. This was intended to reduce the period spent in net-pens, to limit the infestation of salmon lice and thus reduce the need

for treatments. However, site 4 had no license for net-pens, so the first season was designed with a cohort group in net-pens at site 3. The first four CCS at site 4 were ready for use in October 2013; during a heavy storm with full stop in the power supply, the water pressure inside the CCS became critically low. New and improved inlet- and outlet technology had to be developed before it was safe to use the CCS for commercial farming. Next year, in November 2014, site 4 was again operative, successfully producing fish for three year classes until 2017. In 2017, an outbreak of Pancreas Disease at neighbouring sea sites lead to the establishment of a control zone, further sea transfer of smolt was prohibited by NFSA and site 4 had to be abandoned. In 2015, AkvaDesign AS was granted three Research and Development licenses for the further growth of their project. These licenses were used to establish three new sites for the next generation of CCS cages. At the first of these sites (site 5), five new cages (6000 m^3) were stocked with S1 smolt during May-June 2016, and six cages with S0 smolt during autumn 2016. The trials described in Paper II ended in May 2017, while two new 'Development licenses' were granted to the company from 2018.

Along the way, we designed a couple of smaller projects: one study to investigate the impact of production intensity on water quality (Paper III) and one to test the effect of water velocity and water temperature on post-smolt growth and welfare (Paper IV). The study on water quality was performed in two closed cages (2870 m^3) at site 1, from January to September 2014. We recorded water flow, feed use, biomass and water temperatures and correlated this data to pH and concentration of CO_2 inside the cages. The study of water velocity and temperatures was designed as an experimental study with six small CCS cage replicas (volume 40 m^3) (Figure 9). In 2014, we drafted a project together with NORD University, the International Research Institution of Stavanger (IRIS) and NMBU, with funding from the Regional Research Fund, Nord (Project no. 269013). AkvaDesign AS designed the cages and equipment for the first trial at site 1 in 2015: investigating the effect of water velocity on salmon growth and welfare. After completion of the first trial, we tried to implement the next trial with two different temperature regimes. However, the equipment needed for operating two different water temperatures in such large volumes was either unavailable or far too expensive. Because we excluded two test cages from Trial 1, we decided to repeat the trial with water velocity to obtain stronger data. In the first trial, we used fish between 800 and

3000 g; in the next we decided to run the study with smaller fish, 300 to 600 g, located at site 5. We combined the data from the two trials in one article, published in 2018 as Paper IV.

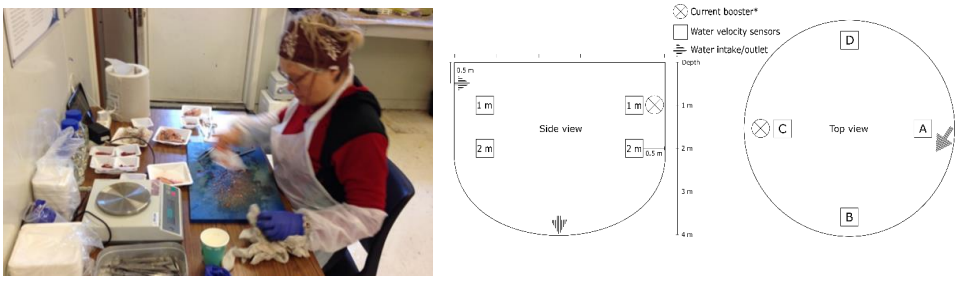

Figure 9. Left: Dr Marit Bjørnevik (NORD University) sampling liver weight during trial 1 (Paper IV). Right: Illustration of the construction of research cages (40 m³), with location of a current booster in the MODERATE velocity cages, arrows indicating location of inlet and outlet, and squares indicating the locations used for measurement of water velocities.

Sea lice counts

With some experience, counting sea lice is relatively easy, and described in detail in a Norwegian best-practice manual from 2013[8]. However, there are some obvious shortcomings to the standard counting protocols.

Detection of chalimii

It is difficult to detect most chalimii and some of the smaller, pre-adult lice without killing the fish and examining the whole surface with magnification. We adjusted for the low detection rate of chalimii by counting two to four times each month during the whole trial period in all cages. Most chalimii would eventually develop into pre-adults and adults, and then, over time, the detection rate should be sufficient.

Representability

With small sample sizes, like the recommended 20 fish in each cage (during 2012, only 10 from each cage were required), representability can be a problem. Sea lice counts often show variation both between cages and within each cage, and this clustering represents a problem when designing counting methods based on independent fish data

[8] http://lusedata.no

(Revie et al., 2005). Heuch et al. (2011) showed that at low and moderate prevalences (<50%), the distribution of sea lice can be described using negative binomial distribution with a linear relationship between prevalence and abundance (more fish with sea lice = more sea lice on each fish). With higher prevalence, the sea lice tend to be normally distributed. Sampling larger numbers (n≥100) from all cages and calculating abundance at site level has recently been proposed as a method for improving the accuracy (Helgesen, pers. com.). We operated with a few research cages and relied on the n = 20 protocol described in the best-practice manual. In the closed cages, all fish are at the same risk of infestation and it is easier to get representative samples than in the open cages, where stratified salinity and temperature profiles lead to different infestation pressure on different fish in the cages. As it turned out, the difference we found was between open cages (moderate to high abundance) and closed cages (no salmon lice). Then the most important corrective measure was to increase the sample size in the closed cages, to avoid conclusions of no sea lice if the truth was abundance below the detection limit of n = 20. We never found chalimii on the fish in closed cages (Papers I, II, IV), and from the duration of the trials (May 2012-May 2017) we concluded that this was a true observation, and not merely a result of low detection rate of these smallest life stages.

L. salmonis and *C. elongatus*

The few *C. elongatus* identified in CCS were adult. They are smaller than adult *L. salmonis* and not easily misclassified (Figure 4). However, under practical counting conditions it could be difficult to differentiate between *L. salmonis* and *C. elongatus*, especially the chalimii. When the abundance of *C. elongatus* is lower than *L. salmonis*, it is more likely that *C. elongatus* are misclassified as *L. salmonis*. In Paper I, we had to revise the counting data from the farm in 2012 and adjust some of the classifications from the net-pens.

Counting bias

Reports from farm personnel could be biased or unreliable. However, a study of the validity of sea lice counts showed no systematic bias when farm staff counted salmon lice compared with dedicated counting teams (Heuch et al., 2011). We also covered most of the cage units with sea lice counts by the research team (Papers I, II, IV) to verify the counts of farm personnel. Altogether, 'zero salmon lice in closed cages' has been

validated by both farm personnel and our research team over years of counting lice on thousands of fish from all the involved sea sites.

Counting fish

To calculate mortality rates, someone, somehow has to count the fish. All fish are counted during vaccination at the hatchery and subtracting the mortality from vaccination to sea transfer should in most cases provide accurate and trustworthy stocking numbers (n_0) (Papers I, II). In some cases, stocking numbers were estimated to the closest 1000. The final number of fish (n_1) was in principal determined by subtracting the recorded mortality from n_0. In cages with moderate to high mortalities the retrieval and counting of dead fish was less precise because large numbers of fish were estimated rather than counted individually. Some fish would also have decomposed and disappeared with the sludge. When the cages were emptied, the fish were moved with well boats and counted. We were informed by the well boat operators that their counters should be within a 2-3% accuracy range, and their counts were also used to evaluate the estimated n_1. At all sea sites, we had to acknowledge that some of the dead fish are never retrieved. Especially during the first period after sea transfer, some of the smallest fish will disintegrate before they are collected and counted. During peaks of mortality, mortalities could also be counted less accurately, as mentioned above. To adjust for these factors, all sea sites used an experience-based correction factor when counting mortalities during the post-smolt period. The actual numbers of counted fish in the large-scale trials were usually multiplied by 2 during the first month and then by 1.5 during the next two months to produce the reported mortality data. Sometimes, this gave us overestimated mortality rates, underestimated estimates of fish numbers, and subsequently overestimation of fish weight. This is not a satisfying procedure seen from a scientific point of view. However, the best way to get the most reliable data from such large-scale trials is probably to make use of the farmers' own experience of how to match stocking numbers, mortality figures and the total count of fish at the end of production periods.

The mortality curves, describing weekly mortality and cause-specific mortality rates, are relative measures describing trends and patterns. In a few cases, these trends are

distorted because of delayed retrieval of dead fish (due to technical problems with the lift-up systems). However, in most cases the data at week level are not as vulnerable to variations caused by repetition of small counting errors as the cumulative mortality data.

Measuring growth

Weight at sea transfer (W_0) (Papers I and II) as reported by the hatchery is also regarded as relatively accurate data. These weights were often verified by sampling fish for individual weight and quality assessment at the time of sea transfer. The reported growth data are combined from reports from the farm databases (spreadsheets or PDF printouts), sampled bulk weights and weight samples from individual fish. Weight samples were performed by farm personnel and/or by the research team. From 11 CCS at site 5, 63 weight samples with >12,000 fish were used to evaluate and adjust the estimated weights in the period from May 2016 to May 2017.

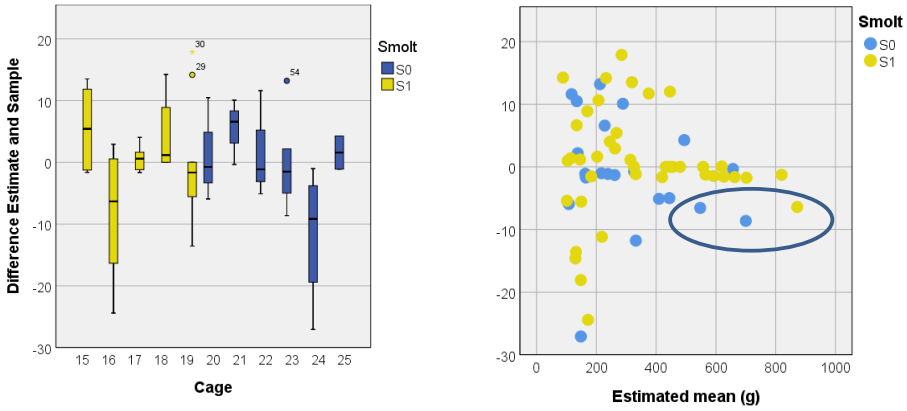

Figure 10. Left panel: Difference (%) between sampled weights and estimated weights from cages 15 to 25, site 5. Yellow bars: one-year smolt (S1), blue bars: off-season smolt (S0). Right panel: Difference (%) between sampled and estimated weights for all weight samples at site 5, plotted against estimated mean weight. Yellow: S1, blue: S0. A circle around the three cages with largest difference between sampled and estimated W_1.

The discrepancy between estimated and sampled weights differed between the cages and weight samples at cage level tended to be largest during the first weeks after sea transfer, while weights were more calibrated towards the end of each production cycle

(Figure 10). In conclusion: the final weights (W_1) were determined using a combination of the farming database and the last supplementary weight controls.

Water flow and water quality

The CCS were supplied with two to four propel pumps (Xylem AS). During the project period from 2012 to 2018, the farming company tried out different pump sizes, propeller types and tube dimensions. They tested equipment for inline measuring of water flow (m^3/min), but this was difficult to calibrate and use under farming conditions. After three years of pilot studies, including the field trials behind Paper III, it became evident that the specifications of the lifting capacity of the pumps were inaccurate. A validation trial of the water flow was performed at site 5 in 2016. By measuring speed at the outlet with a handheld flow-meter (Flow rate sensor, Fybikon AS) and the water flow in the pipeline with a clamp-on ultrasonic flow-meter (Flexim GmbH, Berlin, Germany) across a variety of propeller types (angles) and pump levels, we were able to establish a standard formula for calculating pump level (in Hz) to water flow (in m^3/min). This was used to recalculate the flow in the two test cages at site 1 in 2014 (Paper III) and the 12 test cages at site 4 (Paper II) and to establish a continuous logging of flow for all cages at site 5 (Paper II). Nonetheless, it is necessary to treat these calculated flow data with caution, and more precise in-line flow sensors would have increased the accuracy of the measurements. In particular, inaccuracies in the lower and upper range of flow has recently been evaluated as a cause of model error in Paper III (see Discussion 6.4).

Different water quality parameters show different temporal (during the day and during different production situations) and spatial variations (inside the cages), posing methodological challenges in the design of test protocols. The test sensitivity is also variable between parameters and different sensors or test systems. Laboratory tests could be influenced by the time from sampling to analysis and how samples are stored before analysis. The sensors deployed in the cages (O_2, t) were tested against our calibrated sensors used in the research project and then our own measurements of pH and CO_2 were tested against certified laboratory analysis of pH, CO_2 and alkalinity (all papers). We also repeated parallel measurements of pH and CO_2 to verify the use of pH as the operative parameter for water quality (Paper III). In January 2017, we launched

a separate research project to validate these measurements through a close cooperation with Western Norway University of Applied Sciences (Professor Sveinung Fivelstad). The results from this project were used to test the data presented in Paper III and to convert pH-values to concentrations of CO_2 in Paper II. In 2016, we performed a pilot cross-sectional sampling in the 2870 m^3 CCS cages at site 4 to describe the vertical and horizontal variation of temperature, salinity, dissolved oxygen and pH/carbon dioxide (Paper II). These data were later validated through a more thorough profiling of the 6000 m^3 CCS cages at site 5 (unpublished data). The information we got about the horizontal variation of pH/CO_2 has been used to evaluate data from sampling periods without cross-sectional profiling. In farmed Atlantic salmon, diurnal variations in metabolism with fluctuations in excretion of CO_2 and nitrogenous waste products (TAN) are described (Bergheim et al., 1991; Kvamme et al., 2019). We did not investigate this in depth in our studies. In addition, the data from samples of TAN and suspended solids was too few and inconclusive to support any theories about cage profiles.

Reference groups

Besides the sea lice study (Paper I), the material in the other two descriptive studies (Papers II and III) consists almost exclusively of data from CCS. It could have strengthened the study to include representative reference groups in net-pens. However, this was difficult because of the restrictions of the research and development licenses, allowing only closed cages at most of the research sites. Heavy infestations of drug-resistant salmon lice were challenging in the net-pens, and numerous treatments against lice and forced harvesting of fish with high lice counts represented a systematic bias in disfavour of the net-pens when comparing growth and mortality rates. Further studies with comparison of production in CCS and other rearing systems are necessary. In Paper IV, we compared two groups with different water velocities. We considered the use of net-pen reference groups, but rejected this method because: (1) it would be difficult to describe the velocity and flow patterns in net-pens with the resources and equipment available in the project, (2) the water velocity and flow pattern in small net-pens would anyhow probably not represent the situation in commercial net-pens and (3) the sea lice abundance in net-pens would most likely represent a fish welfare problem.

Ethical considerations

The trials in Papers I to III were conducted with rearing conditions made as optimal as possible and were supposed to reflect the standards of commercial fish farming. The experimental trials described in Paper IV were also based on creating optimal water quality and rearing conditions, to test the effect of different water velocities. The experiments were simulating normal farming conditions and permissions from the Norwegian Research Authority were not required for any of the papers. During the trials, it was still necessary to reflect continuously on the ethics of our research and to take decisions to improve or safeguard fish welfare. Some of the most important welfare issues we were confronted with during the trials from 2012 to 2017 were:

- The impact of variable smolt quality on welfare after sea transfer.
- Stress during transport via well boats.
- Stress and skin lesions after crowding fish at sampling (lice counts, weight controls, welfare assessments) or when transferring fish between cages.
- The management of welfare during periods with increased mortality.
- Increased risk of lesions, stress and mortality with repeated sea lice treatments in net-pens.

The principles of the 3Rs (Replacement, Reduction and Refinement) have been developed over 50 years to provide a framework for performing more humane animal research[9]. In commercial-scale trials, it is not all that relevant to replace the fish used in research, so it became more important to reduce the number of fish exposed to potential harmful treatment (like sampling protocols) and also to refine all protocols to reduce suffering as much as possible. The most invasive research protocol was employed during the growth and water velocity trials in Paper IV. Transferring fish between cages, intensive measuring protocols (to get sufficient data for statistical analysis) and the negative impact on fish welfare from rapid change in cage environment was reflected in increased mortality and lower growth rates in the test groups than their counterparts in the commercial-scale cages.

[9] https://www.nc3rs.org.uk/the-3rs

There were also ethical considerations connected to cooperation between the research institutions and the researchers involved in the trials: e.g. access to data, participation as co-authors, and the possibility of presenting data at conferences or through media. We could have put more effort into drafting written agreements on these issues. However, I am not aware of any serious or unresolved conflicts occurring between our partners during these trials or during the publication of the papers presented in this thesis. On the other hand, we have experienced periods with conflict of interests between some of our commercial partners. This jeopardised parts of the project (Paper IV), but was resolved. To my knowledge and judgement, the use of data in the papers and in this thesis does not violate the contracts and agreements between the research team and any of the commercial partners involved.

Scientific integrity

We were closely associated with this technology project and the commercial partners from the start, sharing office facilities and working together on the sites. Being involved in such projects as a researcher involves two very different modus operandi. The first modus is to participate in everyday monitoring, identifying errors, and suggesting solutions for better fish health and welfare. I have compared this to being the passenger of a high-speed inter-city train. Things move, and they move fast. Any delays in the timetable will be severely punished. The dresin is a suitable metaphor for the other research modus, or what could be seen as more hard-core science. A dresin is slow, encouraging a more relaxed attitude towards both timetables and final destinations, and the slow pace allows for enjoying the view while you are travelling. The drag force of the inter-city train will always tend to divert research activities into what suits the purpose of the high-speed traveller. To phrase it more specifically: there will be a strong bias towards positive results and less enthusiasm towards research activities designed to detect shortcomings or unknown errors in the commercial project. Innovation could be defined as the result of a process that brings together various novel ideas in such a way that they affect society. Our role as independent researchers in such projects is not to deliver 'positive' results, but to investigate and report with as little bias as possible. We discussed this with our commercial partners and established four guidelines, all of

which must be fulfilled to ensure that our research can contribute to the innovation process without negatively impacting our integrity as researchers:

1. High-speed fact-finding projects are a necessary part of the project. They are basically confidential and shall not be shared through reports or publications without careful consideration and agreement between all parties.
2. Long-term and more thorough research for peer-reviewed publications is equally necessary to build a scientific baseline in the project. The immaterial property rights of data material and the possibility of publishing both positive and negative results must be agreed upon by all parties before trials are initiated.
3. Data from research projects could be presented at conferences or through media, both by the commercial partners and the researchers. Each part is responsible for their presentation and opinions. However, research data should never be misused or deliberately misinterpreted to forward commercial interests.
4. To avoid too-close connections between one commercial part and one research institution or between the few people involved, two mitigating measures are necessary. First: research platforms with several research institutions must be established. Other researchers must be involved in the projects, from design and implementation of trials to data analysis and presentation. Second: within the Norwegian Veterinary Institute, the integrity of the researchers involved in the project must be evaluated by their superiors in the institution, and the project activities must be integrated into the overall project portfolio of the institution.

5. Summary of papers I - IV

Paper I – Effective protection against sea lice during the production of Atlantic salmon in floating enclosures

Nilsen A., Nielsen K.V., Biering E., Bergheim A.

Aquaculture 466 (2017) 41-50 (open access). http://dx.doi.org/10.1016/j.aquaculture.2016.09.009

The main driver behind the development of closed containment systems (CCS) has been the increasing problems with salmon lice in Norwegian salmon farms and the concurrent increase in awareness about the potential negative environmental effects of both salmon lice and the treatments used against lice. The aim of this study was to compare sea lice (*Lepeophtheirus salmonis* and *Caligus elongatus*) abundance in CCS with abundance in net-pens. To test this, we monitored 11 CCS and 9 net-pens during three years at four different sea sites. We used a cohort design where salmon of the same origin and size were stocked in CCS and net-pens. At site 1, CCS and net-pens were located side by side; net-pens were also located at the neighbouring sites 2 and 3, while site 4 was only licensed for closed cages. In the closed cages, water was pumped from a 25 m depth, without any filtration or treatment to remove sea lice. No salmon lice were detected in any of the CCS stocked with smolt.

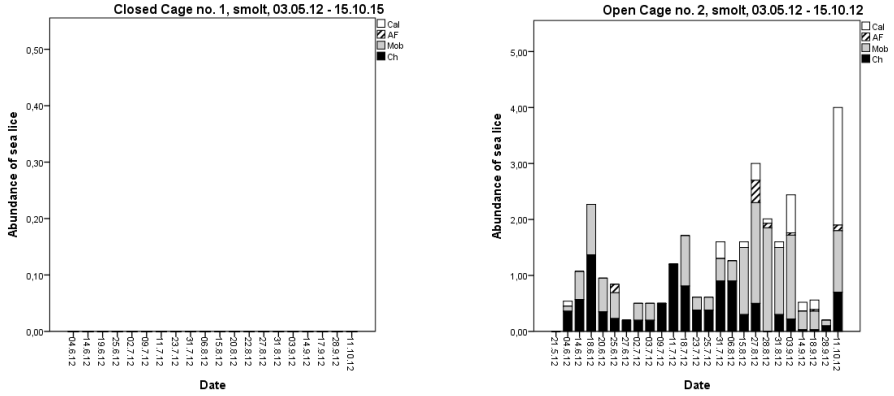

Figure 11. Left: sea lice counts in the pilot CCS, May-October 2012. Right: sea lice counts in the net-pen reference cage. Cal = C. elongatus, AF = adult female L. salmonis, Mob = adult male and preadult male and female L. salmonis, Ch = chalimii

Moderate to high sea lice abundance in reference groups in net-pens confirmed the presence of infective sea lice copepodites in the surface water around the cages. In CCS, adult *Caligus elongatus* were detected sporadically, and with low abundance. Salmon lice (*Lepeophtheirus salmonis*) were recorded in CCS after fish had been moved between cages via well boats, or when the cages were stocked with fish transferred from open cages. The recorded abundance after such incidents was low and we could not find any signs of sea lice reproduction within the cages.

Paper II – Performance of post-smolt Atlantic salmon (*Salmo salar*) in closed confinement systems (CCS): Growth, mortality and rearing conditions

Nilsen, A., Nielsen, K.V., Bergheim, A.

Submitted manuscript to *Aquaculture*, 2019.03.27 (open access)

This study summarise CCS production data from October 2014 to May 2017. We tested 23 CCS and 2 net-pens (the latter for only one season), with more than 3,000,000 fish. The growth rates, mortality rates, mortality causes and rearing conditions are described and evaluated. The mean thermal growth coefficient (TGC) was 2.69 for the two net-pens and 3.79 for the two CCS of the same cohort. For all 23 CCS the mean (SD) TGC was 3.04 (0.37), with minor differences between CCS with one-year smolt (S1, n = 5) and CCS with off-season smolt (S0, n = 18). The good growth rates in CCS could be explained by the aerobic exercise caused by the steady water velocity (13-23 cm/s) and, in the case of the off-season smolt (S0), also by the effect of higher water temperatures caused by the use of water from a 25 m depth. Seasonal variations in growth rates and the possible impact of photoperiod should be investigated further. Mortality rates showed large variations between cages and smolt groups. Cumulated mortality for all fish stocked in CCS was 2.6% after three months and 3.6% after the total trial period (mean number of days was 159). Mortality rates were highest in the S1 cages, with total cumulated mortality of 7.2%, and only 2.4% in S0 cages. At cage level, total cumulated mortality ranged between 0.7 and 10.9%, with a median of 2.1%. The most frequent cause of death, both in the proportion of total mortality (35.3%) and number of cages affected (all 25) was 'Ulcers and fin rot'. The second most frequent cause of death was 'Failed smolt' (18.7%), but this affected only the five CCS with S1. The manifestation of ulcers

and fin lesions during on-growth in seawater was diverse. This reflected the variety of pathogens involved, and the complex interaction between fish, pathogens and the environment. No salmon lice were found in any of the CCS cages during the trial. Water quality and rearing conditions were within recommended standards.

Paper III – The impact of production intensity on water quality in oxygen-enriched floating enclosures for post-smolt salmon culture

Nilsen, A., Nielsen, K.V., Næss, A., Bergheim, A.

Aquacultural Engineering 78 (2017) 221-227. http://dx.doi.org/10.1016/j.aquaeng.2017.06.001

The main aim of the study was to investigate the production capacity of large, closed containment systems (CCS). From the input variables, e.g. water flow, biomass (number and weight), temperature and feeding rate, it is possible to estimate the oxygen consumption, production of CO_2, total ammonia Nitrogen (TAN) and total suspended solids (TSS) and thus establish guidelines for maximum production capacity. Model studies of specific parameters could also be used for the dimensioning of cages and estimation of production limits. However, descriptive studies of observed water quality are necessary to determine how this works in real life on a commercial scale. We used two commercial-scale CCS (2870 m^3 volume) to test the effect of specific water consumption (L/kg/min) and feed load per water flow (g/m^3) on the water quality parameters pH, CO_2, TAN and TSS. The reported production parameters (range) in the two CCS were specific water consumption (q): 0.04-0.47 l/kg/min and feed load per water flow: 9-64 g/m^3. For the water quality parameters in CCS, the range was: pH: 6.8-8.2, CO_2 (mg/L): 1-24, TAN (mg/L): 0.30-1.06 and suspended solids (mg/L): <3-117. We split the study period into two sub-periods: January to May (4.4 -7.5 °C), and June to September (7.5-13.2 °C) before a regression model was used to determine the relationship between production intensity (q, feed load) and water quality (pH, CO_2). With the acceptable level of CO_2 defined as ≤10mg/L, the model predicted a minimum specific water consumption (L/kg/min) between 0.07 (winter) and 0.20 (summer). The predicted maximum feed load per water flow (g/m^3) was between 35 (summer) and 45 g/m^3 (winter). Calculated concentrations of NH3 were <0.010 mg/L. Levels of TSS in CCS were between <3 and 117 mg/L, higher than in the corresponding net-pens.

Paper IV – The importance of exercise: Increased water velocity improves the growth of Atlantic salmon in closed cages

Nilsen, A., Hagen, Ø., Johnsen, C., Prytz, H., Zhou, B., Nielsen, K.V., Bjørnevik, M.
Acuaculture 566 (2019) 50-56 (open access). https://dx.doi.org/10.1016/j.aquaculture.2018.09.057

There are many studies on the effect of water velocity and swimming speed on fish welfare and performance. In closed containment systems (CCS) it is possible to adjust the water flow and water velocity to optimise both water quality and swimming activity. The aim of this study was to replicate the conditions in commercial-scale closed tarpaulin cages (2870 -6000 m^3 volumes) in a model scale system (40 m^3 volume) and to test the effect of the moderate, but stable water velocity observed in the large cages (typically 20-25 cm/s) on a set of outcome variables with relevance to production economy and fish welfare. We used a triplicate design, with 19-21 cm/s as a test group (MODERATE velocity), and 6-8 cm/s as a control group (LOW velocity). In trial 1 we used Atlantic salmon weighing between 884 and 3007 g (168 days, 10.9 °C, mean swimming speed between 0.10 and 0.50 BL/s), and in trial 2 salmon with weight between 327 and 482 g (46 days, 7.1 °C, swimming speed between 0.24 and 0.63 BL/s). The outcome variables were mean round weight (g), length (cm), condition factor (CF), specific growth rate (SGR), thermal growth coefficient (TGC), liver index (HIS), relative heart size (RHS), fillet yield, slaughter yield, mortality rate, fillet chemical composition (% of water, fat, protein), activity of Cathepsin enzymes in muscle and size distribution of white muscle fibres (trial 2 only).

In both trials MODERATE swimming speed (0.36 to 0.63 BL/s) increased fish growth compared to LOW swimming speed (0.10 to 0.27 BL/s). In trial 1, higher swimming speed increased TGC from 2.56 to 2.75, in trial 2 from 2.02 to 2.68. There was no difference in length; thus the condition factor (CF) increased in the MODERATE group. In trial 1, RHS, HIS and fillet yield also increased in the MODERATE group. There were small or no differences in chemical composition of the fillets and no significant effect on muscle fibre distribution (trial 2). We concluded that the increased growth principally had to be a result of increased muscle growth. In trial 1, the activity of cathepsin enzymes in muscle tissue was significantly reduced in the MODERATE group. High levels

of cathepsin activity indicate increased proteolytic activity, possibly mediated by elevated plasma cortisol. Thus, reduced cathepsin activity could indicate reduced primary stress activation over time. This study indicates that MODERATE water velocity and swimming speed have a positive effect on fish growth in closed confinement systems, and that this is probably also accompanied by lower stress and improved fish welfare.

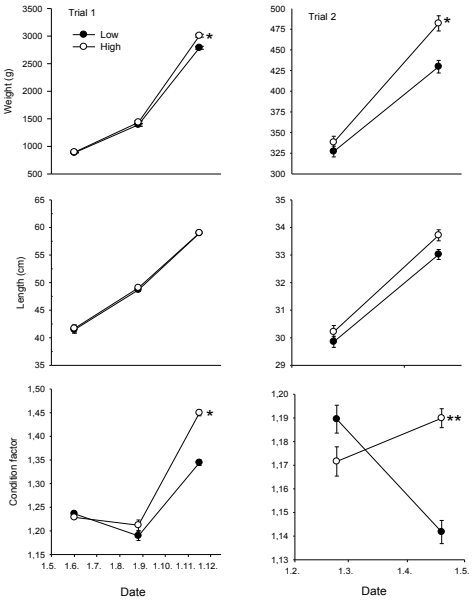

*Figure 12. Mean (SE) weight (g), length (cm) and condition factor in Atlantic salmon exposed to either LOW or MODERATE water velocities in two separate trials (168 days in trial 1, 46 days in trial 2). Significant differences between groups are indicated with: *: p≤0.05, **: p≤0.01 (From Paper IV).*

6. Discussion

The studies presented in the papers describe the production of Atlantic salmon in closed cages (CCS) with emphasis on: (1) prevention against sea lice (Papers I, II), (2) effect on growth rates (Papers I, II, IV), (3) mortality and mortality causes (Papers I, II) and (4) production capacity and water quality (Papers II, III).

6.1 Prevention against sea lice

This was the first and most important issue to settle. At the time when the trials started (2012), no technological solutions were available to remove sea lice from the water pumped into the cages. Regulating depth was the only possible method to avoid infective sea lice copepodites. Site 1 had a record of moderate to high lice counts in the net-pens. The depth was 40 – 60 m and the water flow was dominated by a strong tidal current; periods with both a high infestation pressure and a thorough vertical mixing of water were likely, making it difficult to avoid copepodites. Based on the available data about vertical dispersion of salmon lice (Heuch, 1995; Heuch et al., 1995, Hevroy et al., 2003), we decided to use a 25 m depth for the water intake in the first trials. Without protective roofs or tents, there was also a theoretical risk of contamination with sea lice from waves or from seawater blowing into the cages.

We counted lice in the first CCS with a parallell net-pen every week from May to October at site 1. We found no sea lice in the CCS, while fish in the net-pen were exposed to a continuous infestation pressure throughout the trial period (165 days). At site 1, other net-pens outside the study were stocked with salmon. Salmon in these net-pens showed sea lice abundance and distribution of species and life stages similar to what we observed in the net-pen belonging to our trial. The trial was repeated with a new CCS and net-pen cohort (November 2012-January 2014) (Paper I). We confirmed the results from these sea lice trials through three seasons with S0 smolt at site 4 from October 2014 to May 2017 and with S1 and S0 smolt at site 5 from May 2016 to May 2017 (Paper II). In other CCS projects, the prevention against salmon lice has been variable, as shown in oral presentations at the fifth Conference on Recirculation Aquaculture, Nofima, October 23-24, 2018: salmon lice had been a problem during trials with a 21,000 m^3 composite CCS cage (Trond Rosten, MOWI), low abundance of salmon lice was reported for a 2,000 m^3 raceway CCS (Sigurd O. Handeland, Nofima) and good protection against

sea lice was reported from trials with several smaller CCS models (Per Anders Kvenseth, Smøla Klekkeri og Settefiskanlegg).

Vertical dispersion of salmon lice

Planktonic copepodites respond to light and salinity (Heuch, 1995, Heuch et al., 1995). Both diel vertical dispersion and seeking out haloclines might be host-finding mechanisms. This could be seen as an evolutional adaptation, with vertical dispersion as a possible tradeoff between increased survival and optimal possibility for finding a host (and thus initiating growth towards reproduction). Salmon lice copepodites are heavier than seawater (Bricknell et al., 2006). A swimming velocity of around 0.5 mm/s (1 parasite BL/s) is considered as a reasonable speed for sustained swimming behaviour (Johnsen et al., 2014). In absence of upwards swimming (towards light during daytime), the lice will most probably be mixed by the marine currents. Increased survival for a planktonic organism living on a limited store of energy will largely rely on the ability to grow fast and to avoid predators (Fiksen et al., 2007). Low salinity might increase the energy expenditure for swimming towards the surface or for maintaining osmoregulation (Torres et al., 2002). If the copepodites were only aggregated at the surface, their geographical range of dispersion would most of all be determined by wind and models indicate that in sum, this would lead to a significant reduced horizontal dispersion (Johnsen et al., 2014) and again to reduced infestation possibilities.

Temperature is reviewed as an important regulator of the development of salmon lice (Boxaspen, 2006), but could also be an environmental cue for the behaviour and dispersion of infective copepodites (Johnsen et al., 2014; Samsing et al., 2016). Increased temperature will induce both faster growth and shorter lifetime, but in sum, it will in most cases increase the survival rates of zooplankton like *L. salmonis* because a long lifetime as planktonic prey before attachment to the host is unfavourable. A mathematical model was developed (Johnsen et al., 2014; Samsing et al., 2016) where salmon lice particles were attributed with different characteristics: development time and survival rates at different temperatures, swimming capacity, response to light and darkness and avoidance of low salinity. Giving the sea lice possible dispersion range from the surface to the sea floor, these models were tested across different (historical)

current and weather data from April to August 2009 in the Hardangerfjord, Norway. One model also included temperature-sensitive salmon lice, actively seeking out optimal water temperatures. In short, these models showed different scenarios during winter (cold surface, few sun hours) and summer (warmer surface, excess of light). During winter, copepodites driven by light aggregated towards the surface at a maximum depth of around 16 m, while lice driven by temperature dispersed down to a ≥40 m depth. During summer, when the surface was warmer, the difference between the two dispersion models was reduced, with copepodites driven by light aggregating mostly down to only a 5 m depth, while lice driven by temperature dispersed down to a maximum depth of 16 m. In a study of the same design from 2016, models without temperature-driven behaviour were used (Johnsen et al., 2016). In this study, they focused on the importance of turbulence, finding that salmon lice larvae could be mixed down to and below a 20 m depth. A situation with salmon lice copepodites striving to reach the warmer deep water during winter (mid-September to mid-May) would exclude vertical shielding of any kind as a possible method for reducing infestation pressure in the same period. This is contrary to the results of empirical studies on the use of sea lice skirts (Grøntvedt et al., 2018; Stien et al., 2018), submerged cages (Oppedal et al., 2017) as well as to our results from Paper I, II. Most data support a model where the vertical distribution of salmon lice copepodites is a result of the forces from currents and wind together with the copepodites active swimming behaviour as they seek towards light (diurnal depth variation) or towards a preferable salinity. Unpublished data from Oppedal et al. (2019) [10] summarise salmon lice vertical distribution like this: (1) nauplii and copepodites are phototactic and attracted to the surface during daytime, (2) all larval stages avoid low salinities (brackish water) but nauplii more than copepodites, (3) all larval stages aggregate close to or just below the halocline, (4) nauplii are attracted by low temperatures but no obvious temperature preferences are observed in chalimii. This description of temperature independent vertical dispersion of salmon louse copepodites is supported by our studies.

[10] Presentation at: Norwegian Seafood Research Fund, Sea Lice Conference, Trondheim, January 23rd 2019

Caligus elongatus

Knowledge about the other crustacean parasite, *Caligus elongatus*, is more limited. The life cycle differs from that of salmon lice because *C. elongatus* moults directly from the attached chalimii to adult lice, without any motile pre-adult stages (Piasecki and Mackinnon, 1995). In our trials (Papers I, II), *C. elongatus* was recorded in few samples and at very low abundances. In the trials at site 1, with CCS and net-pens ringside, prevalence and abundance of *C. elongatus* was high in net-pens and only sporadic in CCS. As we never detected *C. elongatus* chalimii on the salmon in CCS, our results indicate that the vertical dispersion of *C. elongatus* copepodites could be similar to what is described for *L. salmonis*.

C. elongatus has a low host specificity and infects several different species of fish along the coast, like lumpfish (*Cyclopterus lumpus*), sea trout (*Salmo trutta*) and herring (*Clupea harengus*), with most fish being infested between May and September (Heuch et al., 2007). *C. elongatus* might have different temperature tolerances and salinity preferences to *L. salmonis*. The fact that adult parasites have repeatedly been found in plankton tows indicates that adult parasites can jump from fish to fish with free swimming in the water as a natural part of the life cycle (Schram et al., 1998). This could be how adult C. elongatus could enter the water intakes and attach to the caged salmon, without any prior detection of chalimii.

Conclusions – sea lice

- Farming of Atlantic salmon in CCS with water intake at 25 m offers effective protection against sea lice copepodites (*L. salmonis* and *C. elongatus*) (Papers I, II). Absence of sea lice and sea lice treatments improves fish welfare and reduce the negative environmental impact from fish farming.
- Sea lice could be introduced into CCS when stocking with salmon from net-pen cages or when moving fish between cages by use of well boats where untreated surface water is used for transport. When sea lice were introduced into CCS, no signs of reproduction or continuous infection were recorded. This could be caused by mate limitation or by effective flushing of any eggs or larvae released into the rearing water (Paper I).

Future research

- Future research should investigate seasonal variations in the vertical dispersion of copepodites (both *L. salmonis* and *C. elongatus*) at different sea sites. This could identify the minimum depth for 'zero sea lice', with the benefit of optimizing (site-specific) intake depths.
- The risk of introducing *C. elongatus* infestations as a result of adult lice swimming into the water inlets could also be tested.

6.2 Growth rates

The data discussed here are reported and discussed in more detail in Papers I, II, IV. Results from net-pen reference cages are used for comparison when possible. However, the main aim with this thesis is to provide detailed data from CCS and to discuss the validity and relevance of the data from our studies for future commercial farming with such systems.

Summary of growth data

In a review of the biological requirements of post-smolt in closed containment systems (Thorarensen and Farrell, 2011), it is suggested that CCS should aim for a thermal growth coefficient (TGC) between 2.7 and 3.0, or even higher than 3.0 in more long-term production or studies. TGC is a growth model validated for use for fish between 100 and 3000 g and for water temperatures between 4 and 14 °C (Alanära et al., 2001). These assumptions were valid for our studies (Papers I, II, IV) and for the studies used for comparison. The only exceptions were a few smolt groups with start weight (W_0)<100 g (Paper I, II) and a few CCS and net-pens (Paper I) where final weight (W_1)>3000 g. From May 2012 to May 2017, we monitored 30 CCS and 9 net-pens, with both post-smolt (≤1000 g) and fish up to harvest size (Paper I, II). We also performed experimental trials on the effect of water velocity on growth rates, muscle development and fish welfare (Paper IV). Mean TGC in the large-scale CCS studies (Papers I, II) was close to 3.0 with positive outliers up to 3.9, confirming the positive predictions of Thorarensen and Farrell (2011). However, the variations are intriguing, and the lessons learned from the less productive cages should be investigated more thoroughly, not least to improve fish welfare. A summary of all data: number of days, mean water temperatures and mean

(SD), median, minimum and maximum thermal growth coefficients (TGC) are shown in Table 1, and growth rates (TGC) in Figure 13.

Table 1. Cage type, fish size (PS = post smolt <1000g, H = harvest size), number of cages, number of days, water temperature (°C), thermal growth coefficient (TGC). Rows 1-2: post –smolt salmon in 25 CCS and 4 net-pens, May 2012 to May 2017, sites 1, 2, 3 (Papers I, II). Rows 3-4: harvest size salmon in 5 CCS and 5 net-pens, sites 1 2, 3, May 2012 to January 2015 (Paper I). Rows 5-8: Water velocity study, 40m³ CCS (Paper IV).

				Days		T (°C)		TGC				
Row	Cage	Fish	n	Mean	SD	Mean	SD	Mean	SD	Median	Min	Max
1	CCS	PS	25	170	60	8.1	1.0	2.98	0.40	2.88	2.24	3.94
2	Open	PS	4	251	122	7.9	1.6	2.86	0.32	2.74	2.63	3.34
3	CCS	H	5	172	97	7.0	1.7	2.99	0.26	3.02	2.62	3.32
4	Open	H	5	227	121	8.0	1.5	3.07	0.67	2.96	2.18	3.84
5	CCS LOW	H	2	168	-	10.9	-	2.56	-	-	-	-
6	CCS MOD	H	2	168	-	10.9	-	2.75	-	-	-	-
7	CCS LOW	PS	3	46	-	7.1	-	2.02	-	-	-	-
8	CCS MOD	PS	3	46	-	7.1	-	2.68	-	-	-	-

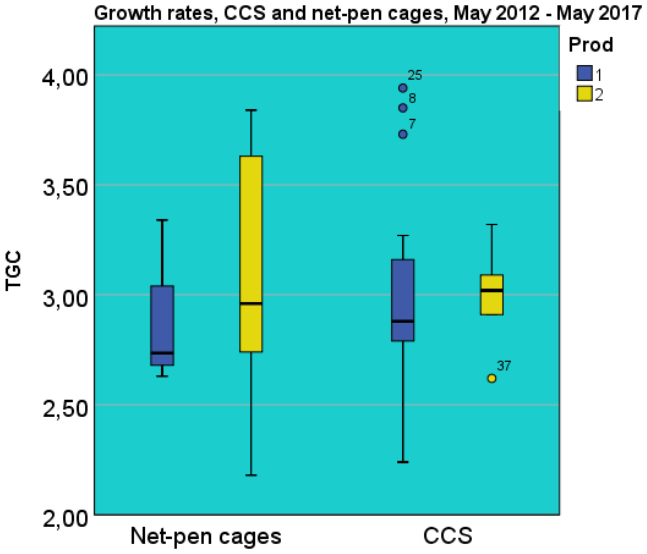

Figure 13. Thermal growth coefficient (TGC) in 9 net-pens and 30 CCS, May 2012-May 2017. Blue bars (1) represent cages with post-smolt (≤1000 g), yellow bars (2) cages with fish up to harvest size.

We observed increased growth rates of post-smolt in CCS compared to net-pens, with the exception of the first two trials at site 1 (Paper I). Data from production of salmon up to harvest size are limited with no apparent differences in growth rates between CCS

and net-pen groups. The two CCS with lowest growth rates (TGC<2.59) were the two first pilot CCS from 2012, with TGC 2.24 and 2.42 (Paper I). Stress during sea transfer (the first cage) and low water flow together with subsequent accumulation of CO_2 and low water velocities (both cages) was assumed to be the principal cause of low growth rates. The quality of all growth data of post-smolt from 2012 to 2013 was hampered by the problems and shortcomings of the systems for regulating water flow in the pilot cages. From CCS with post-smolt (<1000 g), the three positive outliers in Figure 13 were the two cages from the cohort trial with parallel net-pen cages (cages nos. 2 and 4) and cage no. 21 (all described in Paper II). For all three cages a generally good health status at sea transfer, good water quality throughout the trial period and a stable and high water velocity were probably important success factors. I will come back to this in the discussions about water velocity and smolt quality. The data from October 2014 to May 2017 are more homogenous and easier to compare across cages and sites. Figure *14* shows TGC from all 23 CCS in this period (Paper II).

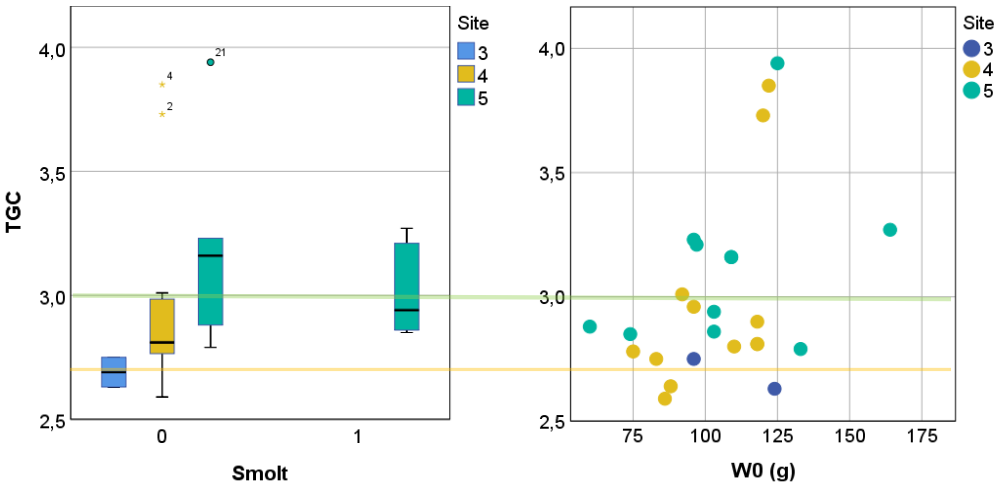

Figure 14. Thermal growth coefficient (TGC) in 23 CCS and two net-pens, October 2014 to May 2017. Left panel: box plot of TGC split into smolt types (S1 and S0) and sites. Right: TGC from each cage plotted against start weight (W_0). Site 3: two net-pens 2014-2015, site 4: 12 CCS, Oct 2014-April 2017, site 5: 11 CCS, May 2016-May 2017. Yellow line: TGC = 2.7, green line: TGC = 3.0.

From these results and the review by Thorarensen and Farrell, I suggest the following standards for evaluating TGC from post-smolt production in CCS:

- TGC≥3.0: good growth rates
- 3.0>TGC≥2.7: acceptable growth rates
- TGC<2.7: suboptimal growth

Growth rates were improved in the 6000 m^3 CCS at site 5, compared to the smaller CCS at site 4 (Figure 14). With higher mortality rates, more ongoing technical development parallel to fish production and a more diverse smolt quality (see: 6.3 Mortality rates and mortality causes) at site 5, this was somewhat surprising. However, there were two possibly relevant differences in the rearing environment: a slightly higher water velocity and higher level of DO at site 5 compared to site 4. With reference to the discussion below about the impact of water velocity and DO on fish metabolism and growth rates, these factors could possibly explain the improved growth rates. At site 5, S0 smolt showed a slightly higher growth rate than S1; this was also probably related to the smolt quality (see 6.3 Mortality rates and mortality causes). The TGC model could possibly also favour growth at low temperatures. The two CCS with suboptimal growth rates (below the yellow line) were cages nos. 7 and 12, both from the 2015-2016 generation at site 4. These two cages were also the two cages with highest cumulated mortality of all CCS cages at this site, with 'Ulcers and fin rot' as the dominating cause of mortality and with a corresponding period with loss of appetite and reduced growth rates. A common denominator for CCS with low growth rates was low mean weight at sea transfer (W_0< 100 g). If a cut-off value of W_0 were to be suggested from this material, it seems reasonable to go for the larger smolt (at least>100 g at sea transfer). Increased W_0 could possibly be related to an overall improved physiological status of the groups at sea transfer (see 6.3 Mortality rates and mortality causes). This should be investigated further.

The combined effects of temperature and day length cause seasonal variations in appetite and growth rates in Atlantic salmon (Brett, 1979; Austreng et al., 1987; Forsberg, 1995; Kadri et al., 1997; Nordgarden et al., 2003). The fish in our trials were exposed to natural photoperiods and fluctuating temperatures. Temperatures in the CCS described in Paper II ranged between 7.0 and 13.1 ºC for S1 smolt and between 5.8 and

12.7 ºC for S0 smolt. As a fun fact about temperatures in CCS cages, we observed that although the temperatures fluctuate with the seasons, there are no temperature gradients inside the cages (Paper II), as opposed to the stratified temperatures that are usual in net-pen cages (Oppedal et al., 2011). In our data material from October 2014 to May 2017, where we had access to weekly data from all cages, SGR increased with water temperature and decreased with increased fish weight, as expected (Paper II). An even more pronounced impact of stocking weight on SFR would probably have been the case if the groups were monitored until harvest size. Water temperatures did not influence TGC in our post-smolt study (Paper II). TGC increased with an almost linear function at stocking weights from 100 to 200 g, but was not influenced by weight between 200 and 1000 g (data not shown). The effect on smolt ranging from 100 to 200 g was probably not an effect of weight or temperatures, but rather one caused by the time needed for the acclimatisation of smolt in the cages after sea transfer. More detailed investigations on these seasonal fluctuations of growth, and probably also fillet quality, would be of interest. Implementation of a growth rate measure including the latitude or photoperiod, like Ewos growth index (EGI) would then be recommended (Aunsmo et al., 2014).

Water velocity

We showed a significant increase of growth and CF with MODERATE water velocities compared to LOW velocities (Paper IV), both for small (300-450 g) and for larger Atlantic salmon (800-3000 g). There was also a significant increase of growth and CF in CCS compared to net-pens in the cohort trial during 2014 to 2015 (Paper II). The results in Paper IV were explained by increased growth of (white) muscle tissue. Moderate exercise of the farmed Atlantic salmon increased body weight and CF, but this was not correlated to increased deposits of body fat. Our data are supported by similar results from recent studies on Atlantic salmon of 80 g size, with several water velocities and a more thorough investigation of muscle histology (Timmerhaus, pers.com.), and by many other studies accounted for in Paper IV. However, other studies of swimming speed between 0.2 and 1.5 BL/s showed no increase in growth rate or CF from 0.2 to 0.8 BL/s and a slight decrease in growth rate at 1.5 BL/s (Solstorm et al., 2015).

In our study (Paper IV), the LOW water velocity was 6 to 8 cm/s, while in the MODERATE group water velocity was 19 to 21 cm/s. This is similar to the difference in water velocity observed between net-pens (3-7 cm/s) and CCS (14-20 cm/s) in the cohort trial from 2014 to 2015 (Paper II). Mean temperature in the cohort trial was 0.2 °C higher in CCS, and it is unlikely that this caused the increased growth rates in CCS compared to net-pens (W_1: 850 vs. 628 g, SGR: 1.16 vs. 0.86, TGC: 3.79 vs. 2.69). There was a strong parallel between the difference in water velocity and the differences in growth and CF in Paper II (the cohort trial) and Paper IV. The final condition factors from other CCS groups (Paper II) were also between 1.18 and 1.29, supporting the high CF observed in the MODERATE velocity group (Paper IV) and the cohort trial (Paper II). There could of course be other confounding variables, but in the absence of any other major health or environmental factors explaining the significant differences in growth, we concluded that the difference in water velocity was an important explanatory variable for increased growth rates and CF in CCS compared to net-pens. It could also be part of the explanation for increased growth rates at site 5 compared to site 4, as discussed earlier.

Weight gain and higher fillet yield (Paper IV) are commercially important effects of increased water velocity. However, water velocity in aquaculture systems is an environmental parameter with a profound impact not only on growth, but also on fish behaviour, metabolism and welfare (Palstra and Planas, 2011), as discussed in Paper IV. Too slow velocity can lead to aggression (Solstorm et al., 2016), and has been linked to longer recovery periods after stressful events (Veiseth et al., 2006). Lactate is produced in muscle tissues under anaerobe conditions, and after a normal oxygen saturation is restored, this lactate could be utilised as an energy substrate in skeletal muscle, heart and other tissues. This effect is documented in mammals and is called the 'lactate shuttle' (Brooks, 2002). A similar effect could also be active in fish, explaining both faster reduction of plasma lactate and faster recovery in exercised fish (Lackner et al., 1988; Jørgensen, 1993). On the other hand, too high water velocities will lead to increased oxygen need and anaerobic metabolism with increased levels of lactate (Davison, 1997, Palstra et al., 2010) and finally to exhaustion, reduced growth and impaired fish welfare (Solstorm et al., 2015; Solstorm et al., 2016). In between these extremes, moderate increase in water velocity has been shown to boost growth rates, increase feed intake, improve FCR, and increase flesh texture and general robustness (all cited in Paper IV).

In our study (Paper IV), we also observed a decrease in levels of cathepsin muscle enzymes with increased water velocity. Increased cathepsin activity is linked to increased intracellular proteolytic activity, possibly mediated by elevated plasma cortisol (Mommsen et al., 1999). Thus, if down-regulation of cathepsins indicate a reduced stress response, this indicates one possible mechanism (of probably many) linking water velocity and swimming behaviour to fish welfare. All in all, there are several good reasons to emphasize water velocity as an important environmental resource with impact on both production economy and fish welfare.

How to provide fish friendly water velocities ?

There are many studies on the swimming performance of salmonids (see discussion in Paper IV). In open ocean studies, the swimming speed approximates 1.0 BL/s, independent of age (Tanaka et al., 2005). In net-pen studies, the swimming speed is rarely corrected for ambient current velocities, this could explain some of the variations in the reported data (Solstorm et al., 2015). As a rule of thumb, Atlantic salmon post-smolts will perform best at swimming speeds of around 0.8-1.0 BL/s, and show signs of exhaustion with velocities>1.5 BL/s with a (temperature dependent) critical swimming speed between 2.1 and 2.7 (Solstorm et al., 2015; Hvas et al., 2017a). A new measure for swimming performance, the preferred swimming speed or U_{pref}, was defined in a study of brook char (Tudorache et al., 2010). For small brook char at moderate water temperatures (26.2±0.6cm, 12.2±0.9^{0}C) the mean preferred swimming speed was significantly lower than the most cost-efficient swimming speed (0.78-0.95±0.03 BL/s vs. 1.02±0.47 BL/s). In addition, during much of their time spent in the research raceway, the char preferred to swim at even lower speeds. The authors suggest that a study of preferred swimming speed (U_{pref}) could be a way to determine welfare-friendly swimming speeds in aquaculture systems. This study has not been repeated for post-smolt Atlantic salmon, but points to the regulation and differentiation of water velocities as an important welfare issue in fish farming.

In open net-pens, the velocities inside the cages are mostly generated by the current velocity outside the cages, moderated by the net (with different mesh size and different levels of biofouling), the biomass of fish inside the cage and the location of the cage with

respect to other cages at the site. In net-pens, the fish have to adapt to several important environmental factors such as light, oxygen levels and access to feed parallel to temperature differences in the vertical water column and regular (tidal) fluctuations of water velocity and current direction (Oppedal et al., 2011;, Johansson et al., 2014). This force the fish to adopt multiple behavioural trade-offs. Nevertheless, few studies have investigated water velocity, swimming speed and fish behaviour in the commercial net-pen farms where most salmon are farmed (Johansson et al., 2014). Salmon farms in many coastal sites in the region where we performed our studies generally experience maximum current velocities below 10 to 20 cm/s with mean water velocity of 5-10 cm/s or even slower (Hagen, L., pers. com.). Our velocity measurements at the research sites showed surprisingly low velocities (<5 cm/s) outside the cages (unpublished data). It is reported that salmon in such net-pens typically swim in circular, one-way schools at speeds of 0.2– 1.9 BL/s, with maximum average values of 1.9 BL/s (Oppedal et al., 2011). With higher water velocities, the schooling behaviour could change, with the fish swimming more against the incoming current than following the circular school (Johansson et al., 2014). With biofouling of the nets, water circulation inside the net-pen will be reduced, the same happens when the cages are covered with skirts to prevent sea lice. From the studies mentioned above, it is reasonable to assume that such reduced water velocities will force the salmon to engage in more active circular schooling. With the extensive use of cleaner fish with a musculature designed for lower swimming velocities than the salmon (Davison, 1988), exposing net-pens to very high current velocities could lead to other fish welfare problems (exhausted cleaner fish).

What about closed containment systems? Again, our knowledge is limited when it comes to use water velocities to create a fish-friendly swimming environment and how to provide good fish welfare. Salmon with weights of 100-1000 g and a condition factor (CF) developing from 1.0 to 1.2 will have a length ranging between 21 and 43 cm. With a targeted swimming velocity of 0.8 to 1.0 BL/s in the post-smolt period, this should be matched with water velocities between 16 and 43 cm/s. An experimental trial with larger salmon (3.4 kg) in net-pens showed how water velocities above 30-35 cm/s disturbed the schooling behaviour, with indications of a forced and potentially stressful swimming behaviour at water velocities above 45 cm/s (Hvas et al, 2017b). In a fixed circular or longitudinal current in CCS (circular or raceways), this effect could be

different. In a raceway-experiment with larger volumes than earlier tests and densities within a commercial range (9-23 kg/m^3), U_{crit} for post-smolt salmon (at 14 °C) of 80, 300 and 1750 g was 4, 3 and 2 BL/s, respectively (i.e. 80, 90 and 100 cm/s) (Remen et al, 2016. High velocity is reported to increase the risk of turbulence (Tvinnereim, 1990) and could thereby also impair schooling behaviour and water quality. In our experience, water velocities>40 cm/s were difficult to maintain in the CCS. Mean water velocities in the largest CCS (6000 m^3) were between 19 and 24 cm/s (with maximum velocities close to 40 cm/s) (Paper II and unpublished data). The absolute water velocity (cm/s) could increase with increased flow rates (m^3/min), but it was difficult to match the increasing weight and length with a simultaneous increase in water velocity as to maintain a swimming speed close to 1.0 BL/s. In our data, the circular, horizontal velocity usually decreased towards the centre of the large CCS, but with insignificant vertical velocity gradients. The same effect is described in studies of land-based tanks (Gorle et al., 2019) and in a CCS simulation model (Klebert et al., 2018). The biomass of fish stocked in CCS will reduce water velocity, as in RAS tanks where the presence of fish reduced the velocity by 25% (Gorle et al., 2018). Data from other CCS studies are scarce, moderate water velocities of 10-20 cm/s (Balseiro et al., 2018) have been reported from a 2000 m^3 raceway system and similar water velocities are described in a simulated CCS model (Klebert et al., 2018). In CCS with uniform and high water velocities, fish with compromised health will seek out sheltered locations (e.g. close to the cage wall or downstream pipes or other installations slowing the water velocity) or they will turn around and start swimming or drifting downstream (our observations). A fast water velocity might be beneficial for growth rates, given optimal conditions. However, we must also take into consideration other needs and welfare aspects. Creating spatial differences in water velocities inside CCS could be utilised to provide an environment suitable for different individual behavioural needs or coping styles.

Feed conversion ratios (FCR) recorded during the trials with one-year smolt (S1) in CCS were considered too low to be reliable (<1.0) and were therefore excluded. Mean (SD) FCR from 18 CCS with off-season smolt (S0) was reported as 1.10 (0.07) (Paper II). More detailed studies on feed consumption and feed efficiency in CCS should be initiated.

Conclusions – growth rates

- Mean TGC in the large scale CCS studies was close to 3.0 with some positive outliers close to 4.0, confirming earlier predictions of growth rates in CCS. There were no noteworthy differences in growth rates between one-year smolt (S1) and off-season smolt (S0) (Papers I, II).
- For post-smolt production (100-1000 g), water velocities around 20 cm/s in CCS improved growth rates and condition factor compared to when salmon were exposed to lower water velocities (<10 cm/s) (net-pens and the experimental LOW velocity CCS). Increased weight and condition factors were not correlated to increased deposits of body fat (Papers I, II, IV).
- We observed reduced growth rates during periods with reduced specific water consumption and accumulation of CO_2 (Paper I). Sufficient water consumption rate is crucial for water quality, fish welfare and production capacity in CCS.

Future research:

- In commercial scale CCS it will be necessary to describe the variation of water velocities and swimming speeds throughout the whole cage volume and identify how the salmon respond to this variation: e.g. growth, oxygen consumption, muscle development and final product quality (according to a function-based welfare approach).
- It should be initiated studies on how create a rearing environment with sufficient temporal and spatial water velocity gradients to allow for the widest possible range of behaviour that could be considered natural for the domesticated salmon (according to a nature-based welfare approach).

6.3 Mortality rates and mortality causes

Cattle die and kinsmen die,
thyself too soon must die,
but one thing never, I ween, will die,
fair fame of one who has earned.

- Snorre Sturlasson, 1200 AD

Hávamál, or the words of Odin, the mythological Norse king of gods, were collected and written on Iceland in the 13th century. The quote above tells us that livestock mortality was a part of everyday life even then. The Norwegian meat industry (Anonymous, 2018d) summarised Norwegian livestock mortalities in 2017: annual mortality rate for dairy cows: 7.3%; beef cattle (first 180 days): 3.9%; piglets until 30 kg: 12%; lambs: ca 11-14% (inaccurate estimates); broilers: 3.2% with 1.7% more condemned at slaughter because of diseases or injuries; turkeys: 5.1% with 3.1% condemned at slaughter. In comparison, the mean annual mortality rate in Norwegian salmon farming between 2014 and 2018 has been 15.2%, with 17.2% for rainbow trout (Hjeltnes, Jensen et al. 2019). Median mortality rate for fish groups from sea transfer to harvest was 15.0% in 2018 (quartiles: 9.0%, 23.1%). Mortality rates do not describe the amount of suffering experienced by animals before they die (Ellis et al., 2012), nor do they account for the impact of the suffering of other fish. In people's awareness, animal welfare of lambs roaming around on green hills and pastures probably outscore the imagined welfare of poultry farms, despite the lower mortality rates in poultry farming. What can the mortality rates from our studies tell us about health and welfare in CCS?

Cumulated mortality rates

Cumulative mortality (CM_{total}) was reported in all papers. In Paper II, we discussed mortality discussed in more detail; in terms of cumulative mortality (CM_{3mo} and CM_{total}), weekly mortality rates and cause-specific mortality rates. Mortality rates vary between cages, between fish groups from different hatcheries, between year classes and from one week to the next (Rodger and Mitchell, 2007; Aunsmo et al., 2008; Soares et al., 2013). In the larger picture, mortality also change between different companies and different

production areas (Gismervik et al., 2019). Mortality data from the freshwater period are less described, but a new project is now launched to investigate mortalities and mortality causes in hatcheries (Brit Tørud, pers. com.). The lack of standardised mortality data across years and countries is an obstacle when discussing mortality rates and cause specific mortality data from salmon farming.

The start weights (W_0), water temperatures, thermal growth coefficients (TGC) and cumulated mortality rates (CM_{total}) from all cages reported in Paper I and II are summarised in Table 2, and CCS are compared to net-pens. Looking at this table, it is also important to remember the methodological problems associated with quantifying mortalities in such commercial-scale trials, as discussed under 'Methodological considerations'.

Table 2. Start weight (W_0) number of days (T), thermal growth coefficient (TGC), cumulated mortality rate (CM_{total}, %) for all large scale trials described in Paper I, II. Post-smolt = salmon ≤1000 g.

			W_0 (g)		T (°C)		TGC		CM_{total} (%)			
Cages	n	Size	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Min	Max
Net-pen	4	Post -smolt	113	13	251	122	2,86	0,32	13,1	14,4	0,8	28,9
CCS	25	Post -smolt	105	22	170	60	2,98	0,40	4,0	4,9	0,7	24,4
Net-pen	5	Harvest	1449	792	227	121	3,07	0,67	5,4	3,4	0,2	8,4
CCS	5	Harvest	1284	607	172	97	2,99	0,26	3,6	3,2	0,3	7,9

The most striking outlier in Table 5 is the mortality rates in net-pens with post-smolt production (Paper I). This was caused by mortality after repeated sea lice treatments in the two net-pens at site 1 and because of 'Ulcer and fin rot' during the second pilot trial. Accumulated mortality in the second CCS trial (S0 at site 1, November 2012-January 2014) in CCS and the net-pen reference cage was 24.4% and 28.9%, respectively.

In Paper II, we described and discussed the mortality rates from the more homogenous data set of post-smolt trials, October 2014-May 2017. We showed a moderate reduction in cumulated mortality rates during the first three months after sea transfer in CCS with S0 (Paper II), compared to net-pen data from 1999-2001 (Åtland et al., 2007) and net-pen data from 2006 (Aunsmo et al., 2008). In Paper II, mortality rates were higher in S1 groups, see more details under 'Mortality causes'. Of the total fish deaths (CM_{total}), 53.2% took place in the 5 worst cages (or in 22% of all cages). Skewed mortality rates are

reported from other mortality studies (Aunsmo et al., 2008; Stien et al., 2018a). High mortalities in relatively few cages could be explained by: (1) all fish in one cage are usually of the same origin, exposed to the same transport stress and environmental factors that could cause or predispose to disease and mortality and (2) the cage is the principal epidemiological unit, and fish inside one particular cage will be at higher risk of infectious diseases (i.e. ulcers and fin rot) through the effect of cohabitation. Low to moderate mortality rates indicated better fish welfare in CCS. However, the volume of data from our trials is limited, the studies cover only a few years and the results should be interpreted with caution.

In 2012, the journal 'Fish Physiology and Biochemistry' issued a special issue on fish welfare. (Ellis et al., 2002) argued that mortality should be used more actively to assess fish welfare. Investigating causes of death during periods with increased mortality will shed light on both immediate and underlying causes of mortality. Long-term or cumulative mortality rates could serve as retrospective welfare performance indicators (Ellis et al., 2002), together with other accumulated biotic parameters such as weight/growth rate and condition factor. Cumulative mortality could also be used to compare different groups when describing a specific period, such as to describe mortality during the first three months after sea transfer (CM_{3mo}) or during the total production period (CM_{total}). Short-term mortality rates like daily or weekly mortality rates (calculated from the number of fish at risk in the specific time period and not from n_0) could be used as a more operational welfare indicator (OWI) (Noble et al., 2018; Hjeltnes et al., 2019).

Weekly mortality rates and mortality causes

During the trials with post-smolt in CCS cages (2014-2017) we used a system of mortality categories (Paper II). The most frequently occuring categories were 'Ulcers and fin rot' (35.3% of total mortality) and 'Failed smolt' (18.7% of total mortality). Both categories were used and interpreted as immediate causes of death, consisting of several exclusive and/or overlapping underlying (proximate) causes. Weekly mortality rates with specified weekly rates for the two most important mortality categories display different patterns across different generations of smolt (Figure 15). Off-season

smolt (S0) at site 4 had low mortality rates after sea transfer, but a moderate increase caused by 'Ulcers and fin rot' occurred from the 17th week onwards. Mortalities in S0 groups at site 5 were low to moderate with a small peak of mortality immediately after sea transfer, without any specific mortality causes assigned. The situation with one-year smolt (S1) at site 5 was something completely different, with a pronounced peak of mortality during the first 4 weeks after sea transfer. Mortality was here defined as a combination of 'Ulcers and fin rot' and 'Failed smolt'. Another period with increased mortality occurred during weeks 13-17 after sea transfer, dominated by 'Ulcers and fin rot'.

Soares et al. (2013), used cause-specific mortality rates to benchmark production data across four generations of Atlantic salmon in Scotland. They reported a high CM_{total} (24%) during 90 weeks of seawater production, and used 52 pre-assigned mortality causes without any information about how this was evaluated at the farms. A more relevant study to compare with our data was published by Aunsmo et al. (2008). The distribution of mortality causes in our study (Paper II) and the net-pen S0 from 2006 (Aunsmo et al., 2008) displayed the same overall trend with 'Ulcers and fin rot' causing approximately 50% of the total mortality (Table 3). Suboptimal smolt quality was the other major cause of mortality in our studies, with cachexia and physical trauma as other noteworthy mortality causes or categories. In the following discussion, I will focus on the two dominating categories in our studies; 'Failed smolt' and 'Ulcers and fin rot'.

Ulcers and fin rot

'Ulcers and fin rot' was the most frequent mortality category and was recorded in all CCS and net-pens in Papers I, II. 'Ulcers and fin rot' also caused mortality in the experimental studies in Paper IV. Mean (SD) weekly mortality rate caused by ulcers (Paper II) for S1 smolt was 0.09% (0.26), and for S0 smolt 0.03% (0.10). The risk of ulcers showed a moderate increase in the last 1/3 of the production period for S0 at site 4 and for S1 at site 5. For S1 groups there was also a more dramatic peak in mortality during the second week after sea transfer (Figure 15). Our unpublished mortality records from Paper I show the same trends in CCS, while mortality connected to sea lice treatments was another important cause of death in net-pens at site 1 and contributed to the higher CM_{total} in net-pens.

Table 3. (From Paper II). Comparison of weight at sea transfer (g), CM3mo and mortality causes in the present trial and a study of 20 net-pens with S0 Atlantic salmon from the 2006 year class (Aunsmo et al., 2008).

	CCS S0+S1	CCS S0	Net-pen S0	National data 2006
Number of fish (millions)	2.8	2.1	2.7	71.1
Number of sites	2	2	10	114
Number of cages (mean no.fish/cage)	23 (122,700)	18 (115,400)	20 (139,700)	667 (103,100)
Species	A. salmon	A. salmon	A. salmon	A. salmon, R. trout
Sea transfer period	08.05 - 21.12	16.10 - 21.12	28.8 - 26.11	1.8 - 31.12
Mean (SD) weight at sea transfer (g)	104 (23)	103 (19)	81 (25.8)	109.7 (43.2)[a]
CM_{3mo} (%)	2.6	1.3	2.1	3.7
CM_{total} (%) (159 days)	3.6	2.4	n	n
Mortality causes (% of total mort.)				
Cachexia	2.4	4.9	3.7	n
Failed smolt	19.3	0	7.4	n
Ulcers and fin rot	36.1	47.5	50.9	n
Trauma	1.3	0	7.3	n
Others	40.9	47.6	30.7	n

[a]Mean weight one month after sea transfer

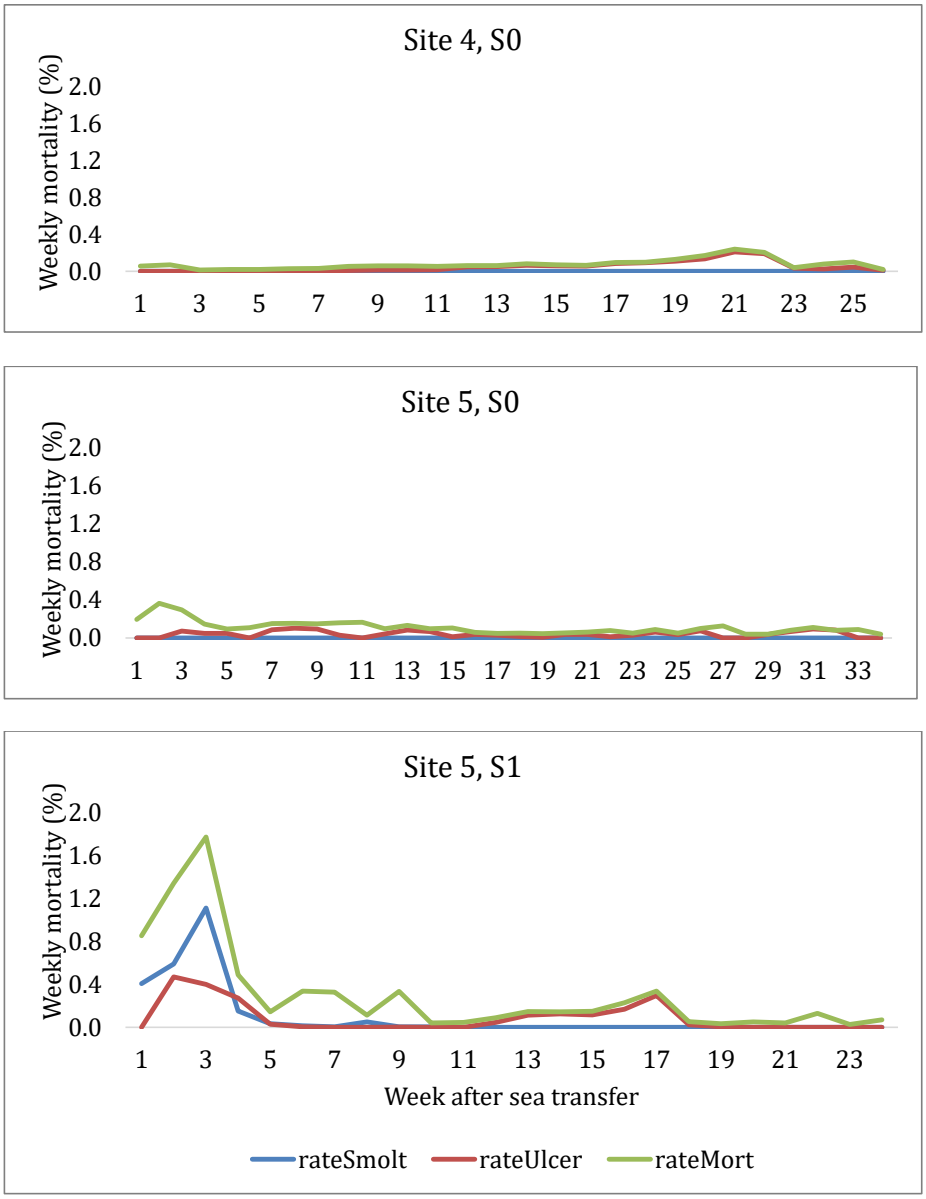

Figure 15. Mean weekly mortality rates from 23 CCS, October 2014 to May 2017. Two sites, S0 and S1 smolt . Blue line: 'Failed smolt', red line: 'Ulcers and fin rot', green line: total mortality. Plotted against week after sea transfer. Data presented in the figure is background material for the tables of mortality causes presented in Paper II.

Predator attacks and mechanical injuries are common risk factors for the development of skin and fin lesions in farmed salmon in net-pens. In one of the experimental units, regular visits by otter (*Lutra lutra*) caused stress and injuries to the extent that all the fish in the cage had to be excluded from the trial. The first CCS cage at site 1 (May 2012) was frequently attacked by herons and sea gulls before an appropriate bird net was installed. Otherwise, predator attacks were of little relevance in the cages during our trials. Absence of salmon lice (Papers I, II) and no potentially harmful treatments against lice (Overton et al., 2018) had a definitely positive effect on fish welfare in CCS compared to net-pens. During the post-smolt trials described in Paper II, no grading or transport of fish between cages was performed, and this reduced the risk of mechanical injuries during these trials. For the CCS with harvest-sized fish, stress and physical injuries suffered during transport between cages contributed to injuries and mortality (Paper I). The same was the case in Paper IV where handling of fish during weighing, tagging and transport to the trial cages was the proximate cause of trauma and skin lesions. Lesions suffered at transport developed within a few days into fin rot and ulcers in the most affected fish. It is important to establish more fish-friendly methods for management procedures like transfer of fish between cages or grading, regardless of cage technologies.

The prevalence of ulcers in Norwegian salmon farms increases with latitude (Takle et al., 2015; Hjeltnes et al., 2019). The common terminology for ulcers caused by these bacteria is 'Winter ulcer', reflecting the connection between ulcer prevalence and water temperature, and thus also the link to sea-sites in Northern Norway. 'Winter ulcer' was first described by (Lunder et al., 1995), who identified two new pathogenic *Vibrio* species, later renamed *Moritella viscosa* and *Aliivibrio wodanis*. According to the annual report from NVI, the bacteria causing skin infections of farmed salmon in seawater in Norway mostly belong to three families and four genera: Moritellaceae (genus *Moritella*), Flavibacteriaceae (genus *Tenacibaculum*) and Vibronaceae (genus *Vibrio*, genus *Aliivibrio*) (Colquhoun and Olsen, 2019). In our studies, culturing from diseased or dead fish showed a variety of bacteria, with high prevalence of *Moritella viscosa* (both 'typical' and 'variant'), *Aliivibrio wodanis*, *Aliivibrio logei,* and to a lesser degree also 'Non-classical winter ulcer' with isolation of *Tenacibaculum* sp. (Colquhoun and Olsen, 2019). Other unclassified but possible pathogenic *Aliivibrio* sp. were also isolated

(Sørum, H., pers. com.). On-going studies of CCS (unpublished data) and the magnitude of *Tenacibaculum* sp. mortality in Northern Norway in recent years (Colquhoun and Olsen, 2019) indicate that this could be an important pathogen to monitor in closed containment systems. Some of these results and the unpublished data described here will be prepared for publication outside this thesis.

For post-smolt with severe fin lesions and ulcers immediately after sea transfer, a combination of suboptimal smolt quality, mechanical trauma during transport and abrupt change in environment were the most likely predisposing factors. For the episodes of ulcers and fin rot occurring in the last 1/3 of the production period (>week 13-17) the situation was different. The skin lesions often started as small areas with loss of scales and petechial bleedings, usually on the side of the body. This could again indicate mechanical trauma as a predisposing factor, but in most cases, these episodes developed without any identifiable traumatic incidents occurring prior to the detection of skin lesions. As we could observe, fin lesions and fin rot usually developed parallel to the skin lesions and with the same bacteria involved. The prevalence of fish with such superficial skin and fin lesions could be moderate to high, but usually only a fraction of these fish developed more severe ulcers and fin rot. When mortalities increased in such groups, close to 100% of all dead fish had developed severe lesions. Episodes of ulcers and fin rot were sometimes accompanied by depressed appetite, reduced growth rates and increased individual variation of condition factors (data not published). The episodes with ulcer and fin rot in CCS during August to September occurred at high water temperatures, but with clinical appearance and bacterial diversity (except *Tenacibaculum* sp.) similar to what we could find during the winter season. Thus, 'winter ulcer' is not necessarily a precise term for all manifestations of this disease.

After transfer to seawater, the epidermis and dermis will be thicker and the number of mucus cells will increase in pace with growth and the time passed since sea transfer (Karlsen et al., 2018b). A gradual morphological development in skin with a delayed recovery of immune functions after sea transfer could increase susceptibility to infections during the early post-smolt period. Sveen (2018) argues in her doctoral thesis that (1) high densities and low specific water consumption activate stress and immune responses in the skin, but do not necessarily produce visible skin lesions, (2) high densities, low specific water consumption, handling stress and inflammations could lead

to reduced secretion of protective mucus and (3) wound healing in Atlantic salmon follow the same mechanisms as for other vertebrates and the effectivity of wound healing is impaired by increased levels of stress. Thus, the risk of developing ulcers in the post-smolt period will depend on both intrinsic (fish response to the new marine environment) and extrinsic (presence of additional environmental stressors) factors. The microbiological diversity in the environment is also important. In a study of the microbiota of water and biofilm in RAS tanks (8-22 ppt salinity) and one CCS (32 ppt salinity) (Rud et al., 2017) observed distinct differences in microbiota between water and biofilms and a temporal change in the biofilm microbiota, with more pronounced change over time in CCS than RAS. In CCS towards the last part of the trial, biofilm contained increasing concentrations of potential pathogenic species including *Tenacibaculum* and *Aliivibrio*.

How then do we interpret our results in light of these data? Ulcers and fin rot caused by bacterial infections seem to be an important health and welfare issue during the post-smolt period. Simultaneous lesions and bacterial infections of the skin on the body and the fins are probably caused by the same pathogens and predisposing factors. These chronic infections lead to increased mortality rates, suppressed appetite, reduced growth rates and condition factors (Paper II). The fish densities in our trials (Paper I, II, IV) were below 25 kg/m^3 and should not represent a risk of compromised welfare or rearing conditions. In the early trials at site 1 (Papers I, III), the minimum SWC was estimated to be as low as 0.04 L/kg/min. At this site, we also observed periods with suboptimal water quality and suppressed growth rates, most likely caused by the low SWC and accumulation of CO_2, suspended solids or other metabolites. However, the minimum specific water consumption (SWC) at sites 4 and 5 (Paper II) was ≥0.20 L/kg/min, with values up to 3.9 L/kg/min at the stocking date. In these trials, the concentrations of CO_2 and ammonia were generally far below threshold levels and the physical water quality should not have represented a risk of compromised welfare or increased susceptibility to bacterial diseases. The microbiological balance in the water and the interaction between fish and pathogens in the rearing environment continue to be important issues for the health and welfare of farmed Atlantic smolt, including both net-pens and CCS. However, as comparable data from net-pens is scarce, in this study it is not possible to evaluate the risk of ulcers and fin rot in CCS compared to net-pens.

'Failed smolt'

Problems with smolt quality are described as one of the most important health and welfare issues in Norwegian aquaculture (Gu, 2019). In our studies (Paper I, II), 'Failed smolt' mortality was only recorded as a significant mortality category in S1 groups at site 5 during the first 4 weeks after sea transfer. This category was defined as: (1) precautious males, (2) parr or discoloured and undersized fish, (3) lethargic or dead smolt with empty stomach and a dehydrated appearance. Deformities is described as a cause of reduced welfare and increased mortality (Fjelldal et al., 2006; Branson and Turnbull, 2008), but the prevalence of deformities in our studies was low, with little effect on the fish welfare or overall growth and mortality rates. The smolt stocked in CCS at site 5 came from two different hatcheries and showed a much larger variation in quality at sea transfer than the smolt we used at site 4. This variation was displayed both as mortality and as delayed onset of normal feeding activity (data not shown). 'Ulcers and fin rot' occurred parallel to mortality caused by smolt quality during the first month after sea transfer. This could be caused by acute bacterial infections, as is the case with *Tenacibaculum dicentrarchi* in land-based post-smolt production (Klakegg et al., 2019), the lesions and infections could also reflect the health and quality of these smolt groups, as discussed earlier. Fish with reduced seawater tolerance could end up as cachectic ('runts') (Gu, 2018). In our trials, few cachectic fish were recorded, except in the first net-pen cage in 2012 (Paper I). Most contracts on sale of smolt allow the sea site to subtract mortalities during the first month from the total number of fish delivered before they pay and the motivation to classify dead fish as 'Failed smolt' is often highest during these first four weeks. Over time, there will also be a gradually reduced validity of assigning mortality to anything caused by smolt quality.

The Norwegian salmon industry has adapted to the use of off-season smolt (Thrush et al., 1994; Handeland et al., 2000, Handeland and Stefansson, 2001), and in 2018, more than 50% of all smolt used in Norway were S0 (Iversen et al., 2018). In our trials (Paper II), 18 of 23 CCS were stocked with S0. Access to warmer (deeper) water during the coldest season (October to April) was seen as an added value to the growth rates and fish welfare of these S0 groups in CCS. The risk of problems with osmoregulation after sea transfer is thought to be higher with the use of S0 smolt (Stefansson et al., 2005). However, improved seawater tolerance and performance has also been shown in S0

smolt compared to different groups of S1 (Lysfjord et al., 2004). Problems with side effects after vaccination and increased prevalence of vertebral deformities are also mentioned as possible problems with S0 production (Stefansson et al., 2005). In our studies, the mortality of S1 was far higher than that of S0, with variable smolt quality as the principal explanatory variable. The challenges with mortality related to smolt quality in S1 groups at site 5 (Paper II) were to a certain degree anticipated from the observed size and quality of the smolt before sea transfer. Smolt quality was evaluated from body silvering and fin darkening (Thrush et al., 1994), seawater challenge tests, Na^+/K^+.ATP-ase activity in the gills and by testing expression of genes involved in freshwater and seawater tolerance (SmoltVision ®). Fin lesions (both healed and active), especially of the dorsal and pectoral fins, were common in the salmon at sea transfer in many of the groups (data not shown). Large differences in fin quality between the smolt groups were also observed, with a striking difference between the different hatcheries. It was not possible to establish one standard smolt protocol across hatcheries and smolt groups, and this was identified as an important opportunity for improvement.

Fish performance after sea transfer depend not only on smoltification status, but also on body size, water temperature and salinity (Handeland et al., 1996; Handeland et al., 1998; Handeland et al., 2008). For S0 smolt, a low and decreasing temperature could lead to increased osmotic disturbance after sea transfer (Virtanen and Oikari, 1984; Sigholt and Finstad, 1990). Reducing salinity from 34 to 28 ppt for post-smolt S0 during the first three months after sea transfer stimulated growth rates at 4 °C, but not at 8 °C (Handeland et al., 1998). In CCS the deep water (25 m) pumped into the cages during winter was salter and warmer than the surface water. Growth rates should be improved by the increased water temperature. However, if the temperature difference between the surface and a 25 m is small, the effect of higher salinity in CCS could possibly be a challenge for smolt groups with an uneven smoltification status. Different combinations of fish size, smolt quality, temperature, salinity and water velocity could possibly have different impacts on fish welfare and performance in CCS during the first period after sea transfer. This should be investigated further.

Another important feature of the smolt groups (Papers I, II) was the prevalence and severity of kidney lesions classified as nephrocalcinosis. This accumulation of mineral

deposits in the tubuli leads to congestion, lesions on tubuli epithelia and finally to more generalised degeneratory and inflammatory lesions in both tubuli and in the interstitial tissue (Rosseland, 1999). Nephrocalcinosis could be triggered by concentrations of CO_2 above 10-15 mg/L (Fivelstad et al., 2003, Thorarensen and Farrell, 2011), or by other mechanisms yet uncovered. Exposure to high levels of CO_2 could also lead to other physiological adaptations, to reduced growth rates in the initial seawater period (Martens et al., 2006) and possibly to increased mortality (Åtland et al., 2007). In our trials, we observed nephrocalcinosis at sea transfer in most smolt groups from 2012 to 2016, with variable severity and a prevalence of up to 50% at sea transfer (data not shown). We performed no studies on the correlation between prevalence of nephrocalcinosis at sea transfer and mortality or growth in seawater. The most serious lesions could prevail during the entire post-smolt period, often diagnosed in individual fish below average weight and CF. Nevertheless, the prevalence of lesions was reduced during the seawater period in both net-pens and CCS, indicating healing of kidney tissue. In addition, we found no signs of continued development of nephrocalcinosis after sea transfer (with the exception of the first two CCS (Paper I) where periods of high levels of CO_2 were recorded). More data on the histopathology of nephrocalcinosis from these studies are under preparation for publication, outside this thesis. From our experience, lesions seem to develop faster in smaller fish and at high water temperatures. Kidney lesions of this magnitude and prevalence at sea transfer are a clear indication of suboptimal fish welfare and represent a risk for reduced performance in the seawater period. The impact of nephrocalcinosis on fish welfare and performance after sea transfer should be investigated in order to develop more systematic scoring systems for macro- and microscopic pathology, determine the most important risk factors and to target necessary interventions.

Smolt is a necessary commodity in salmon farming and contracts for delivery of smolt are often negotiated several years in advance. When doubts about the final quality of smolt groups arise, it might be far too late to implement substantial corrective measures. In companies with in-house smolt production, this could ideally be mitigated by stronger focus on overall productivity and welfare during both freshwater and seawater production periods. If the availability of smolt is restricted during periods of high operating margins (net profit) in the seawater farming, the incitement for quality

grading in freshwater will be reduced if such grading at the same time reduces the number of smolt ready for sea transfer. From 2007 to 2017, the number of produced smolt doubled and the production cost per smolt increased with more than 50% (Norwegian Directorate of Fisheries, 2018a). Production cost per kg salmon increased by more than 75%, and market value increased from NOK 12.2 billion to 61.6 billion. In the same period, mean operating margins decreased for smolt production, while operating margins for seawater production have been undulating in response to the market price for slaughtered salmon, with a temporary peak in 2016 to 2017 with an operating margin around 35% (Norwegian Directorate of Fisheries, 2018a). From the investigations during the trial period from 2012 to 2017 (and now unpublished data from 2018) I got the impression that more effort had been put into increasing the numbers (and weight) than on safeguarding smolt quality and fish welfare. This is matched by the reports of increasing problems with nephrocalcinosis (Gismervik et al., 2019), a disease closely related to production intensity (Rosseland, 1999). However, data from IMR show a steadily improved survival of salmon<2 kg during the period from 2009 to 2016 (Stien et al., 2018), indicating improved survival and an improved quality of smolt at sea transfer. As I see it, it is necessary to implement more studies of health, welfare and mortality in smolt farms.

Conclusions – mortality rates and mortality causes

- Cumulated mortality rates in CCS were moderate to low, compared to mortality rates in comparable net-pen studies (Papers I, II). Together with good growth rates, this indicate a positive fish welfare in these trials (according to a function based welfare approach).
- Ulcers and fin rot caused by bacterial infections was an important health and welfare issue during the post-smolt period at different fish sizes and at different water temperatures. Lesions and bacterial infections of the skin of the body and the fins developed simultaneously and seemed to be caused by the same pathogens and predisposing factors. These chronic infections caused increased mortality rates, suppressed appetite, reduced growth rates and condition factors (Papers I, II, IV).

- Suboptimal quality of smolt at sea transfer increased the risk of post-sea-transfer mortalities (Paper I, II). This was observed as high prevalence of fin lesions, unsmoltified parr and precautious males. During our studies, these problems occurred most pronounced in groups with one-year smolt (S1). A moderate to high prevalence of nephrocalcinosis (Papers I, II) occurred in both S1 and S0, indicating suboptimal rearing conditions at the hatcheries. The correlation between kidney lesions and mortality rates was not investigated. In many smolt groups, a delayed onset of full feeding rates in the first weeks after sea transfer also indicated stress during sea transfer or problems with osmoregulation and thus also reduced fish welfare.

Future research

- All salmon farms should report (standardized) data on cause specific mortality rates and cumulative mortality rates at cage level. These data could be used to evaluate fish health and welfare across sites, companies, regions and years.
- Smolt with insufficient seawater tolerance, nephrocalcinosis and fin lesions represent an unnecessary risk of mortality and compromised fish welfare, across all on-growing cage technologies. Developing more operative smolt welfare and quality indicators are important; relevant interventions before sea transfer should be developed to improve both the fish welfare and the biological output.
- We need a better understanding of the etiology, pathogenesis and possible prophylaxis of ulcers and fin rot. Studies should investigate how the microbial diversity inside the CCS and the temporal variations in load of pathogens are affected by biotic and abiotic management parameters, e.g. the origin of the smolt, intake depth, sea site hydrology, water treatment, season and temperature, fish size, density and water quality.

6.4 Production capacity and water quality

For all trials (Papers I, II, III, IV), the aim was to produce salmon within established thresholds for fish density and water quality. Models of the relationship between water quality and production intensity in CCS are described in Paper III, and the models were used for design of production capacity in CCS for the later trials at site 5 (Paper II). Even though we performed no specific trials on water quality beyond those in Paper III, monitoring and evaluation of fish density, temperature, salinity, DO, CO_2, NH_3 and total suspended solids (TSS) was regarded as important to secure optimal rearing conditions and fish welfare.

Fish density

For closed confinement systems and land-based facilities, there are at present no specific regulations on maximum allowed fish density. For net-pens, the maximum allowed density is 25 kg/m^3. Increased density has been suggested as necessary to increase the biological output from CCS farms with higher investment and running costs than traditional net-pen farms (Rosten, 2011, Calabrese, 2017). Fish density describes the relation between water volume and fish, for practical purposes fish density is often defined by dividing estimated fish biomass by estimated tank or cage volume. The available volume in tanks should be relatively constant; however, net-pen volumes will fluctuate because of deformation caused by high water velocities. As long as water was pumped into the closed cages, we observed no signs of deformation of the flexible tarpaulin bags. Thus, the estimated volumes were evaluated as stable throughout the trials. This happened because the flexible bags were filled up with a water level inside the bags 1 to 2 cm higher than sea level. These extra tons of water are required to keep the bags expanded and since 2013, non return valves and automatic closure of the outlets have prevented emptying or deformation of bags in case of pump failures.

Salmon in net-pens do not distribute randomly, but school in dense groups (Oppedal et al., 2011; Johansson et al., 2014), with a higher density of biomass in the volume actually occupied by fish (Turnbull et al., 2005). Density shows great variation over time, with steadily increasing density due to fish growth until interventions such as sea transfer, splitting, grading or harvesting. The effect of density on fish welfare could be mediated

through reduced water quality, changes in the microbial community inside the rearing units or disturbed social interactions. High densities of farm animals in small confinements have been shown to have a more negative impact on welfare than the same density in groups stocked in larger areas (Andersen et al., 2004). Moreover, the relationship between fish density and overall welfare score is not always linear, some studies report highest welfare with intermediate densities (Adams et al., 2007; Calabrese et al., 2017). The fact that 'intermediate' in one study is defined as 25 kg/m^3 and in the next 75 kg/m^3 points towards the need for a better understanding of the precise mechanisms. Disturbance of the fish is shown to have a significant effect on welfare scores in small scale trials (Adams et al., 2007) and it could be that low densities in research units makes the fish more aware of or stressed by people passing by the tanks, thus explaining reduced welfare at low densities. If low densities lead to reduced welfare, this should be mediated through the quality of the social interactions and how the fish cope with this, rather than by water quality. Fin lesions was shown to arise as a problem in captive fish groups with low density and unstable social hierarchies (Jones et al., 2010; Jones et al., 2011; Jones et al., 2012; Jones et al., 2017). These social network studies showed complex interactions between density, feeding systems, social relations, fin lesions and growth rates in Atlantic salmon, but were performed in very small groups. Density can have different impacts on different welfare indicators (Jones et al., 2011) where an increase in density from 8 to 30 kg/m^3 increased prevalence of fin lesions but at the same time fish in high density groups showed increased growth rate and higher condition factors. These interactions probably exist also in large fish groups, but our understanding of how stocking density affects fish welfare under commercial conditions is not very exact. Precise regulations of stocking density are easy to define and easy to communicate to the public. However, as discussed above, restrictions on fish density are not always the best way to safeguard welfare in fish farming. For freshwater production and marine closed containment systems in Norway 'Fish density should be appropriate and adapted to water quality, fish behavioural and physiological needs, health status, mode of operation and feeding technology' (Norwegian Ministry of Trade, Industry and Fisheries, 2008). An oft-cited study of net-pens in Scotland showed a negative impact on fish welfare when stocking density was ≥22 kg/m3 (Turnbull et al., 2005). However, the technology (30 small steel cages with <25,000 fish per cage) described in the paper is very different from contemporary farming systems in Norway.

In our studies of commercial scale CCS (Papers I, II, III) density ranged between 2 and 25 kg/m^3. Stocking densities in Papers III and IV were also <25 kg/m3. Mean density at sea transfer in CCS (Paper II) was 3.0 kg/m^3, while mean density in commercial net-pens of 160 m circumference and stocking biomass of around 20,000 kg is typically <1 kg/m^3. Within the range of the densities used in our trials, we observed no signs of negative impact of density on fish behaviour (aggression), growth or mortality. Prevalence of fin lesions was mostly observed in the period after sea transfer (low density) and in periods with skin lesions and ulcers associated with bacterial infections (intermediate densities), as described under 'Mortality causes'. Studies on the production of Atlantic salmon in CCS from 1000 to 5000 g at densities of up to 50 kg/m^3 were performed at site 5 in 2017-2018, indicating no negative effect of such densities on growth rates or mortality, given adequate water supply and water quality (unpublished data).

Temperature dependent positioning has a significant impact on schooling densities of salmon in net-pens (Oppedal et al., 2007, Oppedal et al., 2011). In our CCS trials, there were no temperature gradients inside the cages (Paper II) and this could possibly allow a more homogenous utilisation of the cage volumes. Salmon in the CCS trials showed a marked schooling behaviour (Papers II, IV), but we had no means by which to decide the elective schooling densities. We usually observed (data not shown) a circular schooling with avoidance of the volumes close to the cage wall, the water surface and the central vortex. A study of schooling behaviour in 2870 m^3 CCS (Chen, 2015) at site 1 (not reported in our papers) showed minor vertical differences in fish density, but a tendency to denser schooling between 3-6 m depth with large temporal variations. Cross-sectional studies of water quality in CCS (Paper II and unpublished data) showed different linear horizontal gradients of carbon dioxide concentration from the cage wall to the central vortex, but always with insignificant vertical variation (not shown). I evaluated this as a measure of the horizontal distribution of fish (biomass) i.e. schooling behaviour, in the cage at the time of sampling. In some of the trials, especially the earliest CCS versions, the densities close to 25 kg/m^3 were accompanied by low flow rates and thus suboptimal water quality, explaining reduced growth rates and signs of compromised welfare (e.g. nephrocalcinosis, skin lesions and reduced stress tolerance). We did not observe aggression, aggression-mediated fin lesions or suppressed growth rates that could be related to negative social interactions caused by increased density

per se and none of the studies were designed to test any specific effects of density. Thus, there is still a need for more detailed studies on schooling density, elective schooling density, available cage volume and the impact of these variables on fish welfare in commercial scale CCS.

Water quality

We have been working with the impact of production intensity on water quality (Paper III and unpublished data) and in the following; I will discuss the relationship between production intensity and the two most important limiting water quality parameters in CCS: dissolved oxygen (DO) and carbon dioxide (CO_2).

Oxygen and carbon dioxide

Wild Atlantic salmon grow up in rivers and spend their adult life in oxygen-rich surface waters. In aquaculture systems with limited water supply, the level of dissolved oxygen is the main limiting factor for fish metabolism, growth and welfare. Animal cells produce energy when carbohydrates, protein and fat are metabolised in the presence of oxygen, with water, carbon dioxide (CO_2) and ammonia (NH_3) as the principal waste products. Oxygen consumption (MO_2 = mg O_2 consumed/kg fish/min) in salmon increase with decreasing fish size, increasing water temperature, increasing water velocity and increasing feed ration and with pronounced diurnal fluctuations. A model was suggested by Forsberg (1994):

(1) $MO_{2mean}=1.92W^{-0.27}T^{0.63}10^{0.010C}$

where MO_{2mean} = mean daily oxygen consumption (mg O_2/kg fish/min), W = fish size (g), T = water temperature (ºC) and C = water velocity (cm/s). In starved fish, the mean oxygen consumption will be reduced by approximately 50%. In addition, there is a pronounced diurnal variation caused by the feeding schedules, with daily peak oxygen consumption 15 to 25 % higher than the mean values. These models were validated for fish sizes of 0.2-3.5 kg, temperature 6-14 ºC and water velocity of 15-50 cm/s (0.3-1.0 BL/s). An exponential increase in oxygen consumption with increasing temperature has been described, explaining why the linear formula from Forsberg is valid only at low to moderate water temperatures (e.g. 6-12 ºC) (Remen, 2012). When calculating oxygen

consumption using the data from our studies (temperature=6-13 ºC) it will suffice to use formula (1). Studies or models covering higher temperatures should adjust for a possible exponential increase in MO_2.

A practical definition of hypoxia is the level of DO at which it is possible to detect negative effects on feed intake and growth rates. Suggested hypoxia limits for salmon post-smolt are between 70% DO (for temperatures≤16 °C) (Remen, 2012) and 85% DO (Thorarensen and Farrell, 2011). If oxygen levels continue to decrease, salmonids will respond with compensatory mechanisms e.g. increased breathing rate and increased heart stroke volume (Randall, 1982), increased rate of anaerobic metabolism (Remen et al., 2012) and initiation of stress responses (Bonga, 1997). The oxygen concentration where these responses are activated is defined as the limiting oxygen concentration (LOC) (Remen, 2012) and could be used as the threshold between moderate and severe hypoxia. According to Remen (2012), the estimated or adjusted LOC ranged between 40% DO (6 °C) and 60% DO (13 °C). The exponential effect of high water temperatures and the presence of large individual differences between fish of the same species and size should always be considered.

In our studies, the oxygenation was mostly based on a continuous supply of oxygen gas from a net of perforated tube diffusors, located at 8-12 m depth. Each cage was originally supplied with one oxygen sensor, located in the periphery of the cage, to avoid being 'fooled' by high oxygen saturation in the water above the diffusor nets. Sensors were very sensitive to biofouling and after the first test periods, two sensors were deployed to detect drift of sensor readings. At sites 1 and 5, 85% DO was used as the lower set point, at site 4, 70% DO was used. At both sites, 100% DO was used as the upper set point. With low density and high water flow, the need for extra oxygen supply is low, but with increasing weight (and biomass) the oxygen used inside the CCS will become increasingly dependent upon the infused oxygen. Mean oxygen level at site 4 was 81%, below the suggested DO minimum of 85% (Thorarensen and Farrell, 2011), at site 5 mean DO was 86%. The measured DO in CCS fluctuated between 71% and 131%, above the suggested LOC threshold and below levels believed to represent a risk of acute toxic effects on salmon (Thorarensen and Farrell, 2011). The oxygen values in net-pens were higher, indicating that these net-pen sites were of good quality with respect to the balance between biomass, feeding rates and water flow. Unpublished data from 2017-

2018 indicate that more cross-sectional studies on DO are needed to optimise the location of sensors and reduce temporal and spatial variation of DO inside the closed cages. From a function-based welfare approach, it is absolutely necessary to keep DO above LOS thresholds and also beneficial to regulate DO to values above the moderate hypoxia thresholds. Reduced growth rates could be the outcome of both low oxygen saturation and large fluctuations (Remen, 2012). From a nature-based welfare approach, it would make sense to try to keep DO as close to natural levels (up to DO of 100 %) as possible.

Given sufficient oxygen supply, accumulation of CO_2 is the most important limiting water quality parameter in both land-based tanks and closed cages (Sanni and Forsberg, 1996), unless the used water is efficiently aerated. Carbon dioxide is a water-soluble gas produced through the oxidative metabolism of fat, protein and carbohydrate and then released from fish blood across the gill epithelium (Randall, 1982). In water, CO_2 is rapidly engaged in a series of chemical reactions, leading first to the formation of carbonic acid, then to bicarbonate and the release of H+ and thus to a sinking pH. Elevated PCO_2 in plasma is also associated with increased plasma cortisol, changes in the hydro-mineral balance, nephrocalcinosis and tertiary stress responses, e.g. reduced growth and reduced feed conversion ratio, reviewed by Fivelstad (2013). In high concentrations, dissolved CO_2 has anaesthetic effects or becomes lethal (Bernier and Randall, 1998). In wild marine fish, increased levels of CO_2 are also associated with impaired olfactory function, explained by effects both on the olfactory system and the central brain functions (Porteus et al., 2018). Acceptable maximum levels of CO_2 in tank or cage water has been suggested up to 20 mg/L (Good et al., 2018), but also with evidence of a safe maximum level as low as below 10 mg/L (Fivelstad et al., 2003; Thorarensen and Farrell, 2011; Fivelstad, 2013; Fivelstad et al., 2018). In our studies, we used a CO_2 threshold of 10 mg/L as a guideline for implementing interventions like increasing flow rates, reducing feed rates or preparing for splitting the biomass to new cages, and setting 15 mg/L as the maximum allowed CO_2 concentration.

For practical purposes, the diffusion of CO_2 from seawater to the atmosphere during a retention time limited to around 200 minutes is probably negligible (Asbjørn Bergheim, pers. com.). It seems fair to use the maximum levels (the diurnal variation) as limiting values for SWC and water quality. Because a large proportion of CO_2 in seawater is

transformed to HCO_3^-, it is also proposed to measure the difference between outlet and inlet of total carbonate (CT) to assess the real production of CO_2 (Kvamme et al., 2019). In any case, when evaluating fish welfare measured CO_2 levels are probably the most relevant estimate for the effect of water quality. In our papers and in this discussion I refer to the measured levels of CO_2 (or levels calculated from pH and alkalinity). Most measurements were taken in the early afternoon, at which time, both theoretically and according to our empirical data, the oxygen consumption and thus levels of carbon dioxide should have been highest.

During the trials in Paper III we made several relevant observations: (1) accumulation of CO_2 to levels above 15-20 mg/L seemed to have a negative impact on fish behaviour and appetite, (2) with maximum levels of CO_2 around 10-15 mg/L we never reached harmful levels of TAN or NH_3, and (3) it was difficult to obtain accurate and reliable records of feed consumption, oxygen consumption and specific water consumption in these large CCS. After field trials in 2016 with validating of the water flow (see 4. Methodological considerations), it was possible to revisit the data from 2014 and develop new and more reliable models. However, the challenges with estimation of water flow were only partly resolved. With larger cage volumes and inlet pipes throughout 2016 and 2017, we discovered new possible shortcomings of our water flow estimates.

The models we proposed in Paper III were based on empirical data only. The model predicting pH and CO_2 from specific water consumption at summer temperatures (7.5-13.2 C) (from Paper III, cited below as equation (2)) was the model with best fit to our sampled data.

(2) $CO_2=\exp(3.371-7.980\cdot SWC+0.061\cdot t)$

where SWC = L/kg/min and t=temperature (ºC). However, new trials with evaluation of production intensity and water quality during 2017-2018 (unpublished data) show some deviation from this model. If we use values representative for the CCS study (t = 9 ºC, density = 22.3 kg/m3, weight = 3 kg and water velocity = 15 cm/s) and calculate the predicted water quality with the model for oxygen consumption from Forsberg (equation 1) and a model for the relationship between oxygen consumption and carbon dioxide production (Brett, 1979), the mean concentration of CO_2 would rise to 10 mg/L

when SWC is reduced to 0.14 L/kg/min. Calculating from the maximum (diurnal) oxygen consumption rate, a specific water consumption of 0.17 L/kg/min would lead to a concentration of CO_2 of 10 mg/L. With our model (equation 2), the minimum SWC needed to keep the concentration of CO_2 below 10 mg/L would be 0.2 L/kg/min. These data are also under preparation to be published outside this thesis.

The relationship between production intensity and water quality was described in Paper III. The limits for minimum specific water consumption (SWC) and feed load (FL) that we described in Paper III were within a range described in several other studies on salmon metabolism and water quality in flow-through systems. Specific water consumption and the maximum feed load are important measures of production intensity, regardless of fish density. As for fish density, these parameters will change throughout the production cycle and they will define the carrying capacity of the cages. When density increases with increased fish weight, water flow must be increased to meet the demands of waste removal. Recommended SWC from other CCS studies is 0.3 L/kg/min (Thorarensen and Farrell, 2011; Calabrese, 2017). In a survey of production of Atlantic smolt and post-smolt salmon in large tanks, (Summerfelt et al., 2016) present data from 55 land-based tanks (7 sites) and one floating CCS. Their data on specific water consumption and feed load are presented together with data from our studies in the next figure. Our data match the survey of land-based tanks, with exception of the extreme values (outliers denoted with a red circle) of feed load>60 g/m3 and SWC<0.05, both from the first CCS trials at site 1. The CCS reported in the survey by (Summerfelt et al., 2016) (grey circle) was tested at a much lower production intensity.

The data from Summerfield et al. (2016) are close to the rearing conditions in Norwegian hatcheries (1999-2001) described by Åtland et al. (2007). A recent study from a raceway CCS (Balseiro et al., 2018) reports a maximum density of 33.4 kg/m^3 and an estimated minimum SWC of 6 L/kg/min, which is far more than the SWC shown in the figure above. Figure 168 shows how the feed load values from our studies (except from the early outliers) were in the same range as in large land-based flow-through tanks. The models we calculated from feed load (Paper III) were less precise than SWC models, but feed load is a very important production parameter in both land-based farms and CCS. Estimation of oxygen consumption and production of CO_2 is necessary to design production capacities in CCS, given good estimates of flow rates, biomass, fish

size, temperature and water velocity. During the pilot trials described in this thesis, it was often a challenge to establish reliable flow rates. These studies should be continued.

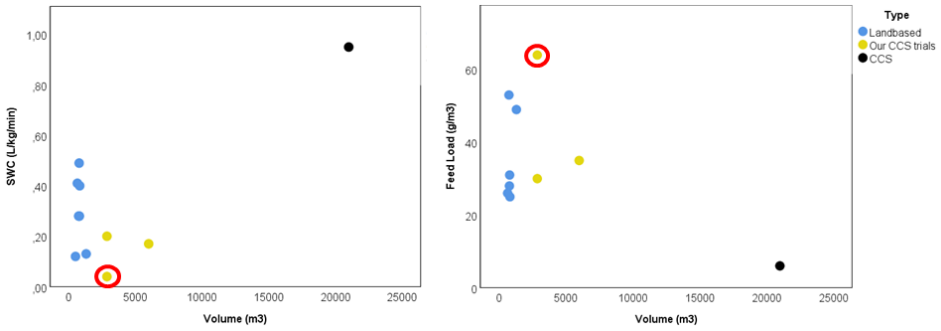

Figure 16. *Specific water consumption (L/kg/min) and feed load (g feed/m^3) plotted against tank/cage volume. Blue: Land-based tanks, Black: 21,000 m^3 CCS (both from: Summerfelt et al., 2016). Yellow: CCS studies described in Paper I, II. Red circles: outlier data from Paper I.*

Ammonia and suspended solids

The other important waste products from fish metabolism and digestion of feed are ammonia (NH_3) and suspended solids (faeces). Our studies of these parameters were not detailed enough to support any models of temporal or spatial variation during production of salmon in CCS. For both parameters, there is a considerable variation in suggested threshold levels for salmonids. For ammonia, we used 0.012 to 0.0125 mg/L (12-12.5 µg/L) as the threshold (Fivelstad et al., 1995, Timmons et al., 2001). With oxygenated water without aeration, the minimum SWC recommended to keep ammonia below these concentrations is 0.05 L/kg/min (Thorarensen and Farrell, 2011), far below the recommended 0.2-0.3 L/kg/min. In our data from Papers I, II (not shown in detail in the publications), moderate production intensities typically led to a TAN between 0.5 mg/L and 1.2 mg/L. Because increased CO_2 and reduced pH will increase the proportion of TAN present as NH_4^+ (Forsberg, 1995), the increased metabolic impact on water quality is not followed by an increased concentration of toxic NH_3. Water samples form the most intensive production periods in CCS (low SWC) showed levels of TAN and pH representing concentrations of NH_3 towards 0.010 mg/L (all data not published). Our studies of total suspended solids (TSS) have been inconclusive, and often confounded by high plankton counts and particle density in inlet water. In large,

circular tanks, experiments have shown that a secondary, radial bottom current with water velocity>6-8 cm/s is necessary to effectively remove sedimenting particles (Tvinnereim, 1990). This would require a circular primary water current with velocity>12-15 cm/s. With the design of the CCS studied in this thesis, the circular primary current velocity was calculated to be 15-25 cm/s, and this was also validated by our studies (Paper II and unpublished data). Current velocities with efficient particle removal should facilitate separation of the largest particles, leaving them to sediment towards the cage wall and sink down to the sedimentation chamber before the water leave through the main outlet. Water samples taken from the outlet often proved to contain fewer particles than water from the inlets, indicating an effective sedimentation of particles in the CCS. With the production intensities (density, SWC, feed load) investigated in Papers I, II and III, we usually found little evidence of accumulated levels of suspended solids in the rearing water in CCS, compared to net-pens. However, in the trials described in Paper III, TSS in CCS ranged between <3 and 117 mg/L, compared to between <3 and 13 mg/L in the net-pens. The effect of suspended particles on salmon health and welfare has been identified as an important welfare issue in RAS; and we need more information about the variations of TSS in CCS and on how episodes with moderate to high loads of TSS could affects fish health and welfare.

Water quality and fish welfare in CCS

From the water quality studies in Paper II (unpublished data), DO at 20-30 m depth was down to 85% in the late autumn, rising to 100-110% during the spring from March to May. In a study of Norwegian hatcheries, (1999-2001) mean DO of inlet water was 133% (16.4 mg/L), and supersaturation was necessary to manage production with median densities of 43 kg/m^3 and median SWC of 0.29 L/kg/min (Åtland et al., 2007). During most of the production period with post-smolt in CCS, the oxygen needed for fish metabolism had to be supplied by adding extra oxygen to the inlets or the rearing water, and oxygenation systems with automatic feedback regulation and alarm functions were a vital part of the cage technology in the CCS described in this thesis. Thus, the oxygen concentrations and water temperatures reported for CCS in Papers I, II and III showed little risk of hypoxia compared to the situation described in commercial net-pens

(Vigen, 2008; Remen, 2012). The theoretical models for oxygen consumption and production of carbon dioxide discussed above appear to be relevant for the dimensioning of production of post-smolt Atlantic salmon in CCS, with the exception of the need for more precise models of oxygen consumption at water temperatures above 12 ºC. However, all suggested minimum values of DO% and maximum values of CO_2 should be viewed with extreme caution. Atlantic salmon in seawater are fast-swimming, pelagic fish with high growth rates and oxygen demand, and with a higher sensitivity for hypoxia than many other fish species (Bickler and Buck, 2007). In salmon farming, it is important to avoid severe and recurring hypoxia. Future research should not aim narrowly at identifying the maximal biological input and minimum standards of life conditions for the farmed fish. It should be equally important to study how farmed salmon respond to more optimized and high-quality environments.

The neuroendocrine and physiological stress response in teleost fish have large structural and functional similarities to that of terrestrial vertebrates, including evidence of interactions between the neuroendocrine system and the immune system (Bonga, 1997; Conte, 2004). However, there are also important differences to acknowledge. Most terrestrial and aquatic animals maintain similar plasma osmolarity and ionic concentrations. Freshwater fish are exposed to a hypo-osmolar environment and seawater fish to a hyper-osmolar environment. This has led to different strategies for exchange of water and ions between the fish and the environment (Takei, 2000). In fish, a stress response will increase the permeability of surface epithelia (gills and gut) and thus induce systemic hydromineral disturbances (Bonga, 1997). Because this immediate interaction with the surrounding environment is inherent to stress in fish, environmental factors such as water pH and mineral composition also have a significant impact on stressor intensity. When we discuss rearing conditions and fish welfare in CCS, the focus is often on a few parameters at a time, e.g. specific water consumption (SWC) vs. CO_2 and pH or oxygen levels (DO%) vs. growth performance. These simplifications are useful when evaluating non-complex interactions between fish and the environment. However, addressing the full depth of how fish deal with more complex environmental cues or stressors is also important. Here lies a possible fallacy when establishing specific thresholds for environmental parameters. Under environmentally-driven rearing conditions, such as those in net-pens, there is a limited

possibility of manipulating basic environmental parameters and for the most part farmers have to rely on local ecosystem services. In land-based facilities and in CCS it should be easier to optimise the rearing conditions, but unfortunately it is also easy to fail, i.e. to produce a mismatch between the needs of the farmed fish and the rearing conditions provided in tanks or cages. Here lies a vast number of possibilities for further research and development.

Conclusions – production capacity and water quality

- From our studies, recommended minimum specific water consumption (SWC) during production of post-smolt Atlantic salmon (weight<1000 g, density≤25 kg/m3, 6-13 ºC) is 0.2 L/kg/min. Recommended maximum feed load (same assumptions) is 35-40 g feed/m^3 (Papers II, III). These values should be interpreted with caution.
- DO between 71% and 131% was recorded in CCS, with mean values between 81% and 86% (Paper II). Mean values were close to values described as optimal for the growth performance of farmed Atlantic salmon. The (short-term) extreme variations were above the threshold for severe hypoxia (LOC) and below toxic levels (all papers).
- Carbon dioxide (CO_2) concentrations were mostly below the threshold for negative impact on welfare and growth performance (10 mg/L) (all papers), but with a few cases of CO_2>15 mg/L, where we also observed a negative impact on appetite and welfare (Paper I). Restrictive limits on levels of CO_2 was evaluated as important to secure productivity and fish welfare in CCS.
- With SWC>0.2 L/kg/min and CO_2<10-15 mg/L, calculated concentrations of ammonia (NH_3) should be below the threshold for negative impact on growth and fish welfare (0.012-0.0125 mg/L). Water samples from different production intensities also showed levels of NH3<0.010 mg/L (Papers II, III; IV).
- Total suspended solids (TSS) were difficult to monitor and evaluate in CCS systems because of high seasonal fluctuations in the inlet water and the relatively efficient sedimentation of large particles inside CCS (Paper I, II, III). However, in CCS with low SWC, the concentration of TSS could be high.

- With the use of deep water, seasonal variations in temperature were reduced and temperature stratification inside the cages was eliminated (all papers). From a nature-based welfare approach, avoiding extreme temperatures should improve both growth rates and fish welfare. However, eliminating the possibility to choose temperature could also be negative from a nature-based approach.

Future research

- We need more detailed studies on schooling density, elective schooling density, available cage volume and the impact of these variables on fish welfare in commercial-scale CCS.
- Documentation of trials with salmon>1000 g and densities of 25-50 kg/m^3 in CCS could be necessary to evaluate production capacity and fish welfare during more intensified production of salmon up to harvest size.
- The fluctuations of TAN/NH_3 and TSS in CCS and how this affect fish health and welfare during periods with high production intensity should be more thoroughly investigated.
- Trials in commercial scale CCS should investigate the outcome of providing the farmed salmon with as fish friendly rearing environment as possible, e.g. stabilising levels of dissolved oxygen closer to 100%, increasing SWC and reducing water retention time with the aim of minimising the impact of carbon dioxide, ammonia and TSS.

7. Concluding remarks

How wonderful that we have met with a paradox. Now we have some hope of making progress.

- Niels Bohr

In 2018, both industry and scientists were criticized for how we describe farmed salmon and the fish that dies during production in terms of 'biomass' rather than as individual animals (Gismervik et al., 2019). It was suggested that the term 'biomass' should be replaced with 'fish' or 'salmon', and that the term 'loss in production' would be more specific if divided into terms such as 'dead fish', 'escaped fish' and 'unidentified loss of fish'. Phrasing such as '15 out of 100 salmon died from trauma or infections within the cages' is arguably much more likely to arouse concern than language such as a '15% loss per generation during production in seawater'. Animals dying from trauma, environmental hazards or infectious diseases will most likely experience some kind of suffering before they die. We observe this in fish farming as loss of appetite and changes in swimming behaviour until lethargy and death. We could argue that fish recovering from trauma or disease are lucky to survive, but it is again fair to assume that they have also been through a period of impaired welfare. Thus, recording mortality will reflect only a part of the total impact on fish welfare represented by the identified mortality causes. In my opinion, this is the reason why recording and analysing mortality in fish farming (or anywhere) can never be merely a descriptive activity. When we record cause-specific mortality rates, we observe and measure suffering through the lens of a fish pathologist. However, these numbers and proportions must also be evaluated in terms of the total welfare on both a group level and for the individual fish involved. We are obliged to use and understand mortality data together with all available information in order to improve fish health and fish welfare.

As I see it, the principle of the 3Rs (Replace, Reduce, Refine) could be an incitement for replacing small-scale trials with large-scale trials and protocols based on intensive measurements of live fish with more non-invasive measurement protocols. Animal-based welfare indicators at group level (growth and mortality rates) and resource-based welfare indicators (environmental parameters) should be utilised to reduce the number

of fish exposed to potentially harmful sampling procedures. Refined methods such as camera vision and software to monitor weight and length (and condition factors) could reduce the need for stressful and potentially harmful crowding and netting procedures. Individual recognition, lice counts and surveys of ulcers and other external lesions are within reach thanks to today's technology. The tagging of individual fish for the purpose of longitudinal, individual data sampling is obviously a risk for compromised welfare and thus also reduced validity of the research data; tagging should thus be restricted as much as possible. To establish a national database on mortality and mortality causes is one of several very easy, non-invasive and fish-friendly methods for improving our understanding of fish health and welfare in commercial fish farming. The commercial database software programs used by all commercial farmers are also powerful tools where the integration of basic resource-based welfare parameters such as temperature, oxygen, CO_2/pH, water velocity and specific water consumption could be linked to animal-based observations such as growth, condition factors, mortality and appetite and improve our day-to-day understanding of fish welfare.

8. Perspectives for the future

During the discussion of several crucial topics in this thesis, a common phrase has been: 'our knowledge is limited'. This should not be very surprising, as the task was to describe a new cage technology in salmon farming. On the other hand, salmon farming is a large and still fast-growing industry in our part of the world and I am often taken aback by how new growth is envisaged without first addressing basic biological and ecological knowledge gaps related to this particular form of industrialised animal husbandry. However, my hope is that this thesis will contribute towards improving and refining our understanding of what we really do not know about salmon farming in both closed cages and other rearing systems.

Some of the important research topics that should be investigated in the near future are:

1. Sea sites, hydrology and water quality necessary for large CCS farms.
2. Flow pattern and particle removal in CCS.
3. Smolt quality. The physiological requirements for smolt or post-smolt used in CCS.
4. Microbial diversity in CCS, the effect on fish health and welfare. How to establish 'healthy' microbial communities.
5. Fish welfare; how fish cope within the CCS environment, behaviour and physiology.
6. The environmental impact of CCS, from local impact on sea sites to LCA and carbon footprint analysis.

As a final admonition, I urge the fish farming industry to execute more corporate sustainability responsibility. The total environmental impact of industrialised salmon farming must be reduced. The buck has to stop somewhere; most investments and operating costs associated with higher environmental standards should be covered by the industry. And importantly: the bill should not be passed on to the farmed salmon, as in the case with thermal delicers where high mortalities and low fish welfare was the price paid to remove lice without drugs. The role of the authorities and the development of new legislation around salmon farming is crucial in this process. Encouraging environmentally and fish welfare-friendly fish farming technologies could be an idea to explore.

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10. Scientific papers I-IV

I

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Effective protection against sea lice during the production of Atlantic salmon in floating enclosures

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ABSTRACT

Effective protection against sea lice (*Lepeoptheirus salmonis* and *Caligus elongatus*) was documented over three years during the production of Atlantic salmon (*Salmo salar*) in floating enclosures with water intake at 25 m depth. Moderate to high sea lice abundance in reference groups in open cages confirmed the presence of infective sea lice copepodites in the surface water around the cages. In the closed cages, sea lice were only recorded after fish had been moved between cages with well boats, or when the cages were stocked with fish transferred from open cages. When fish were exposed to sea lice in the closed cages, the recorded abundance was low and with no signs of sea lice reproduction within the cages. Records of mortality and growth during the test period indicate that production in closed sea cages is possible without adverse effects on survival or growth rates.

Statement of relevance: This study demonstrates how a new closed confinement technology provided an effective protection against sea lice (*L. salmonis* and *C. elongatus*), without adverse effects on survival or growth rates.

1. Introduction

The two sea lice species, *Lepeophteirus salmonis* and *Caligus elongatus*, are copepod ectoparasites found on salmonids in seawater (Boxaspen, 2006; Pike and Wadsworth, 1999). They live and reproduce on fish, but spread by the release of eggs into the seawater. These eggs hatch and develop into planktonic infective stages (Costello, 2006). *L. salmonis* is often referred to as the salmon louse because it is specific to salmonids, especially Atlantic salmon (*Salmo salar*). *C. elongatus* is less host specific and has been collected from 80 different species (Boxaspen, 2006). Commercial fish farming in open net cages leads to increased numbers of susceptible hosts, and thus to increased reproduction and spread of parasites. This is both a threat to the affected fish farms (Costello, 2009) and to wild fish populations living in the coastal areas (Taranger et al., 2015; Torrissen et al., 2013).

The Norwegian salmon industry has experienced increasing difficulties with salmon lice (*L. salmonis*) (Norwegian Food Safety Authority, 2014), including increased resistance against the most important chemoterapeutants (Jansen et al., 2016). Norwegian authorities have imposed severe regulations (Directorate of Fisheries, 2012), and further growth of salmon farming in Norway is restricted unless the levels of salmon lice are controlled. *C. elongatus* is also reported to cause skin lesions on Atlantic salmon (Tørud and Håstein, 2008), but due to its broad host range and more sporadic occurrence this species is not included in the Norwegian surveillance and eradication programmes.

Treatment to control lice infestations in Norwegian salmon farms increased from 2013 to 2014 (Jansen et al., 2016) and represented in 2014 an estimated average cost of 2-5 NOK per kg produced salmon, or 9–23% of the total production cost per kg salmon (Iversen et al., 2015).

Transfer of production from sea to on-shore sites or production in closed, floating sea cages have been suggested as a possible solution to the problems with sea lice. Production in closed confinement systems may also result in better controlled rearing conditions and more effective production (Thorarensen and Farrell, 2011), collection and use of solid waste (Braaten et al., 2010) and a higher level of biosecurity due to treatment of inlet water. Experimental studies have shown that the planktonic stages of *L. salmonis* disperse in the water column, and that their location is influenced by factors such as diffusion (Johnsen et al., 2014), light (Heuch et al., 1995), swimming activity (Heuch and Karlsen, 1997) and salinity (Bricknell et al., 2006; Heuch, 1995). A model study (Johnsen et al., 2014) argues that, if nauplia and copepodites react first to light and salinity, the safe depth of water intake could be below 10 m during summer and below 15 to 20 m during winter. If the temperature is the factor deciding vertical movement, a

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safe depth would generally be below 20 m, but with a risk of finding nauplii down to >40 m during the winter season. Several studies of different cage technologies have shown that restricted contact between salmon and surface water could give reduced infestation levels, as with submerged cages (Stien et al., 2016) (Korsøen et al., 2012) or skirts that enclose the upper parts of open pens (Grøntvedt and Kristoffersen, 2015). *C. elongatus* is less host specific, has a similar life cycle to *L. salmonis*, but without the mobile stages on the host (Piasecki and Mackinnon, 1995). For this study, we assumed that the vertical distribution of infective *C. elongatus* could be within the same range as for the *L. salmonis* copepodites. To date, effective sea lice control over longer time periods in closed sea cages has not been demonstrated.

The main aim of this study was to document the effect of closed cage technology on sea lice abundance by comparing floating enclosures to open pens. The underlying hypothesis was that the vertical distribution of infective sea lice (*L. salmonis* and *C. elongatus*) is restricted to depths <25 m. Closed cages with water intake-depth at 25 m were used as test units. To obtain conclusive results, production was followed for three years, with closed cages located at two different sea sites, one with a strong coastal current and one sheltered site inside a narrow fjord. The monitoring of growth and mortality was included, and these results will be briefly described.

2. Methods

2.1. The cages

The closed cages with necessary equipment such as floating systems, tarpaulin, pumps, inlet, outlet and oxygenation systems were produced and patented by Akva-Design AS (www.akvadesign.com) (Fig. 1). The impermeable tarpaulins were suspended in floating buoys made of separate elements. The cages were circular with a circumference of 62–70 m. From May to October 2012 (Cage no. 1), the tarpaulin had a volume of 1550 m^3 and depth of 9 m, and from October 2014 to May 2015, the tarpaulins had a volume 3000 m^3 and depth of 12 m.

In this study, it was decided to use a fixed intake-depth of 25 m. This decision was made as a trade-off between avoiding infective sea lice copepodites, gaining access to warmer water during the winter season and the technical limitations of the construction (pipelines, pumps, stability). The hypothesis was that supply of inlet water from 25 m, even without filtration or disinfection, should provide sufficient protection. This could be counteracted by water currents transporting infective sea lice to deep water, wind or waves contaminating the cages with spray of surface water, by seasonal biological changes of sea lice reproduction and dispersion, or by unforeseen technical problems. During the pilot period, it was necessary to make several technical adjustments and changes. Such attempts increase the risk of contact with surface water contaminated with sea lice larvae.

The closed cages were supplied with 2 water pumps (2.7–5.5 kW, Xylem Norway AS), each with a maximum theoretical capacity from 10 $m^3 \cdot min^{-1}$, used in the 1550 m^3 cage in 2012, to 20 $m^3 \cdot min^{-1}$, used in the 3000 m^3 cages during the rest of the project. The water was pumped from 25 m depth and pushed into the cages 0.5 m below the surface, to avoid the extra energy cost of lifting water above sea level. The water was pumped into the cages without any filtration or other treatment, apart from a gross filtration (mesh size: 25 mm) to keep out fish, diving birds or sea mammals. The water level inside the closed cages was 2–3 cm above sea level, corresponding to an extra weight of 6-11 MT. This was necessary to push the water out from the cage and to maintain tarpaulin shape and volume. The specific water consumption (Q) was estimated by recording the pump frequency (Hz) and measuring the lifting height (cm) in the inlet tubes, using a conversion table from the cage manufacturer. Sedimentable particles (faeces and surplus feed) and dead fish were collected and pumped in separate tubes from the outlet to the surface. To prevent escapees the entire tarpaulin with outlet and pipelines for sludge and dead fish was covered by a standard fish net (not shown in the figure).

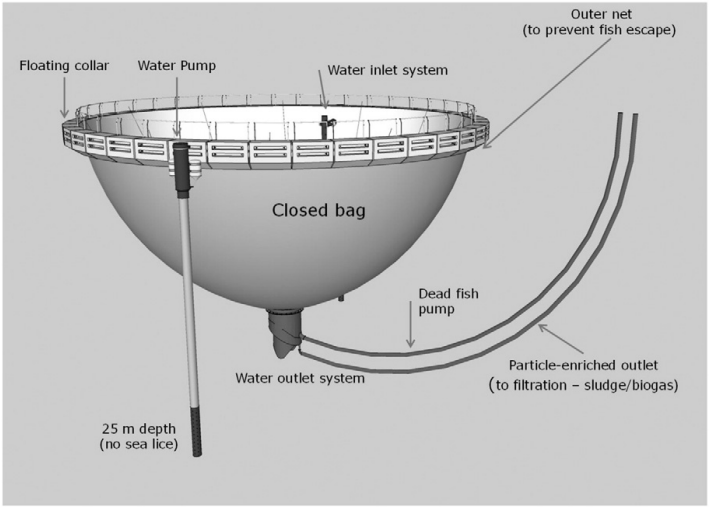

Fig. 1. Design of a closed, floating tarpaulin covered cage. Water inlet at 25 m depth through a 25 mm filter. Effluents separated in three fractions: water, sludge and dead fish. A net (not shown in the figure) surrounded the cage and the tubes to prevent escapees. (Illustration: Akva Design AS).

Oxygen was injected inside the closed cages by a continuous supply of oxygen through a net of perforated tubes (Akva Design AS) suspended 1 to 2 m above the outlet. The oxygen level was logged in each closed cage with a combined oxygen/temperature/salinity sensor (IQ Sensor Net). Temperatures were recorded daily by sensors connected to the farm data systems (AkvaFarm AS, IQ sensor net). Supplementary registrations of temperature, oxygen, salinity and pH were made by the use of a handheld multimeter (SmarTROLL MP, Tormatic Inc.) with corresponding software InSitu app (InSitu Inc.). CO_2 was measured with an OxyGuard portable CO_2 analyser (Sterner Aquatech AS), or calculated from the measured pH values (Moran, 2009). In open cages, the water temperature recorded at 4 m depth was used as an estimate of the average water temperature in the cage.

The open sea cages were commercial standard circular nets suspended in buoyant tubes with a circumference of 70 to 160 m (Polarcircle AS, Aqualine AS) with volumes from 5600 to about 30,000 m^3. The nets were cylindrical tubes with a cone-shaped bottom.

2.2. Sea sites and fish

Four sea sites were used in the project (Fig. 2). The closed cages were located at Sites 1 (Picture 1) and 4 (Picture 4), reference groups in open cages at Sites 1, 2 and 3. The timing of infestation and the abundance of sea lice differ between sea sites (Heuch et al., 2011). Therefore, we stocked reference groups and test groups at the same site when possible. As a second option, cohort groups in open cages of a commercial

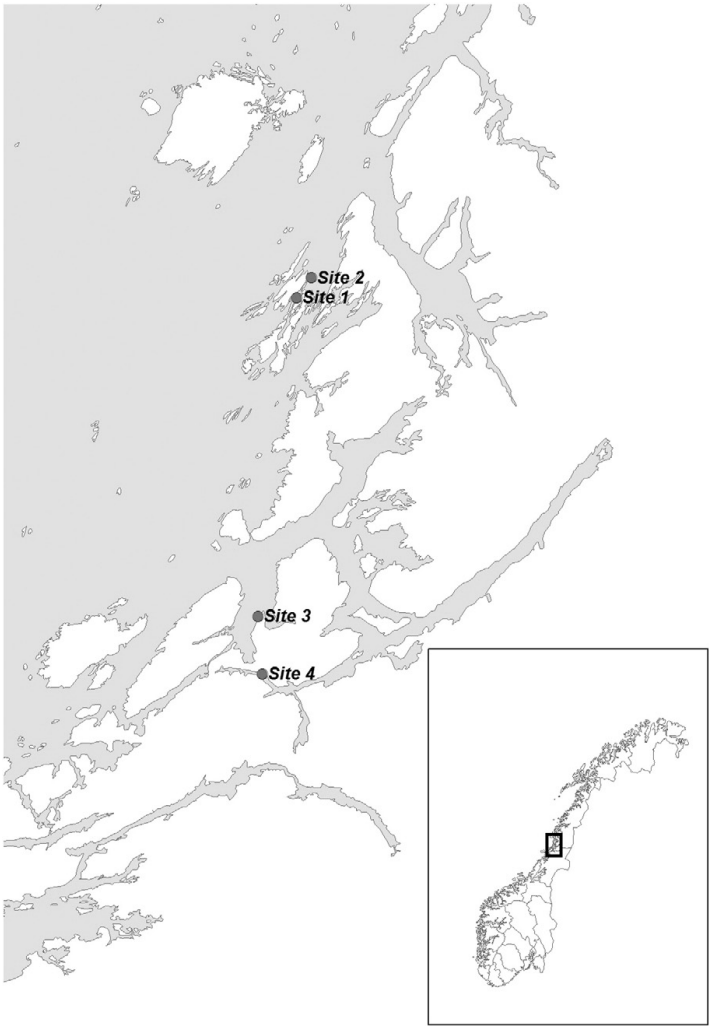

Fig. 2. Location of sea sites in Brønnøy and Bindal, Nordland county, Norway. Site 1: research site with closed and open cages, Site 2 and 3: commercial sites with open reference cages, Site 4: research site with only closed cages. (Illustration: A. Tarpai).

standard on sea sites close to the research sites were used as a reference. Site 1 was the only site licensed and equipped to use both closed and open cages, and the reference groups there were the most representative parallels. This was a research site (maximum allowed biomass 300 MT), located in a strait with strong tidal current and without any well-defined seasonal thermocline or halocline. At stocking date, the density of smolts in the closed cages was low (1.7–5.3 $kg \cdot m^{-3}$), but when density reached 25 to 40 $kg \cdot m^{-3}$ the cages were emptied and the fish harvested or moved to open cages or other closed cages. Because of the biomass restrictions at Site 1, some of these groups were moved to open cages at Site 2 or Site 3. Site 2 was a commercial site (maximum allowed biomass 4680 MT) located 2 km north of Site 1, and with the same coastal environment. Site 3 was a commercial site (maximum allowed biomass 5080 MT), located on the coastline south of Site 1 and 2, with an expected coastal temperature and salinity profile similar to Sites 1 and 2. In 2014 a new research site, Site 4 (maximum allowed biomass 600 MT), was established close to Site 3. This site was located in a fjord, close to the hatchery, and the National Food Safety Authority (NFSA) licensed Site 4 only to use closed cages. Thus the reference groups for Site 4 had to be located at site 3.

The fish were Atlantic salmon (*Salmo salar*, of the Norwegian Salmo breed), all from the same commercial hatchery (Bindalssmolt AS). All groups were fed commercial pelleted feed according to the farms' standard operating procedures. The fish were fed at the surface by automatic feeders. To reduce possible confounders caused by differences in fish size or genetics, our study included cohorts with the same origin, identical light regime and smoltification time, and transferred to seawater at the same date. During the project from May 2012 to May 2015, five cohorts of salmon in a total of 20 different cages were included: 11 closed and 9 open cages (Table 1). The closed cages were stocked with 10,700 to 86,895 fish (size range 85–4850 g). The open cages were stocked with 15,500 to 166,700 fish (size range 120 - 5300 g). In total 445,781 smolts were delivered directly from the hatchery to 6 of the 11 closed cages (Cages no. 1, 6, 17, 18, 19, 20). The other closed cages (Cages no. 3, 8, 9, 12, 13) were stocked with post-smolts, moved from closed cages or with post-smolts moved from an open cage (site 3). The start date was the day of stocking the cages, closing date when emptying the cages (harvesting or moving fish).

2.3. Counting sea lice

Sea lice were counted on anaesthetised or recently killed fish, and the results of this study are reported as mean abundance in each sample. The numbers of lice on each fish were recorded by trained personnel at the farm or by the research personnel (Heuch et al., 2011). The following four categories were used: (1) *L. salmonis* and *C. elongatus* chalimus (attached to the skin), (2) *L. salmonis* adult males and preadult males and females, (mobile lice), (3) *L. salmonis* adult females and (4) *C. elongatus* adult stages. Adult *L. salmonis* females may be as long as 10–15 mm, often with long egg strings attached, and are easy to identify. Adult stages of *C. elongatus* are smaller, the females are 5–6 mm in length. Female *C. elongatus* with egg strings are easily identified, but males and females without egg strings are more difficult to differentiate from preadult stages of *L. salmonis*. If viewed with magnifiers they can be recognised by their characteristic lunules on the frontal segment (Piasecki and Mackinnon, 1995), but this is seldom possible on live fish under field conditions. Chalimus stages of *C. elongatus* can be identified by having a longer and more slender frontal filament than *L. salmonis* (Boxaspen, 2006). However, this differentiation is also difficult and all chalimus were recorded as one group. The chalimii of *C. elongatus* develop into adults without any preadult stages (Piasecki and Mackinnon, 1995). Fish can be infected by both adult and copepodid *C. elongatus* and it has been suggested that *C. elongatus* can be transferred to farmed salmon from passing schools of wild marine fish, leading to immediate infestations on farmed salmon (Revie et al., 2002). The knowledge of the epidemiology of *C. elongatus* is limited, and the parasite is considered as less dangerous to both farmed and wild fish than *L. salmonis*, but because these two species are common on farmed salmon in Norway both species were included in this study.

The Norwegian regulations on counting and eradication of salmon lice must be followed at research sites as well as at commercial farms (Directorate of Fisheries, 2012). These regulations identify the maximum acceptable sea lice abundance, recommended method of sea lice counting and suggested treatment strategy. In 2012, sea lice were counted on 10 fish from each cage; from 2013, 20 fish were counted from each cage. All cages should be monitored weekly or every second

Table 1
Group size, time periods, survival rate (SR %) and growth (Thermal Growth Coefficient) from 20 cages with Atlantic salmon, monitored in the project period from May 2012 to May 2015. Four different sea sites, 11 closed cages compared to 9 reference groups in open cages, five different smolt groups (cohorts) all from the same hatchery. N_1 = number of fish stocked in each cage, t = number of days, T = average temperature in °C.

Cage no.	Table no.	Site no.	Cage type	Cohorts	N_1	Start date	Closing date	t	T	Survival (SR %)	Growth (TGC)
1	3	1	Closed	1 Smolt	80,000	03.05.12	15.10.12	165	9.4	97.1	2.2
2	3	1	Open	1 Smolt	20,000	03.05.12	15.10.12	165	10.2	78.3	2.7
3	4	1	Closed	1 Post-smolt	13,350	15.10.12	12.09.13	332	6.7	92.1	3.0
4	4	1	Open	1 Post-smolt	17,054	15.10.12	30.08.13	319	6.4	99.8	3.8
5	4	2	Open	1 Post-smolt	62,500	15.10.12	08.11.13	389	7.8	91.6	3.0
6	5	1	Closed	2 Smolt	80,000	04.11.12	17.01.14	439	7.1	75.6	2.4
7	5	1	Open	2 Smolt	20,000	04.11.12	10.01.14	432	7.7	71.1	3.3
8[a]	6	1	Closed	2 Post-smolt	10,700	17.01.14	10.07.14	174	6.1	94.3	3.1
9[a]	6	1	Closed	2 Post-smolt	27,300	17.01.14	22.04.14	95	4.9	99.7	2.9
10	6	3	Open	2 Post-smolt	22,775	17.01.14	25.06.14	159	6.6	92.8	2.2
11	6	1	Open	2 Post-smolt	27,224	22.04.14	01.08.14	101	9.4	92.5	2.7
12	7	1	Closed	3 Post-smolt	33,194	30.04.14	01.08.14	93	7.9	99.2	2.6
13	7	1	Closed	3 Post-smolt	18,545	01.08.14	16.01.15	168	9.2	96.9	3.3
14	7	1	Open	3 Post-smolt	17,832	01.08.14	16.01.15	168	9.7	96.1	3.6
15	8	3	Open	4 Smolt	164,700	24.10.14	16.05.15	204	6.8	99.2	2.6
16	8	3	Open	4 Smolt	166,700	24.10.14	16.05.15	204	6.8	99.1	2.6
17	8	4	Closed	4 Smolt	56,365	19.11.14	16.05.15	178	7.3	98.5	3.6
18	8	4	Closed	4 Smolt	57,010	19.11.14	16.05.15	178	7.3	98.8	3.6
19	8	4	Closed	5 Smolt	86,895	19.11.14	05.05.15	176	7.3	98.7	2.7
20	8	4	Closed	5 Smolt	85,511	19.11.14	06.05.15	168	7.3	99.1	2.8

[a] In cages 8 and 9 the fish was moved between the units.

week, but this was not always implemented. If water and air temperature were extremely low or other factors made it undesirable to handle the fish, the sampled number of fish was reduced or sea lice were not counted.

It is difficult to investigate low sea lice abundance in large fish groups (Heuch et al., 2011; Jimenez et al., 2012) and several corrective measures were implemented: (1) at low abundances most fish will have no lice, and to document zero levels it is necessary to increase sampling size or the observation time. The cages were inspected regularly with counts of 10 to 20 fish from each cage. Periodically the sample size was increased (≥50 fish from each cage), (2) the chalimii stages and small preadult lice are difficult to identify because of their size, and these groups are most likely underreported. If the groups are monitored over time as mentioned above, chalimii stages will develop into larger, preadult stages and finally into large and easily observable adult stages, (3) the handling of fish with crowding, dip net and anaesthesia will increase the possibility for some of the preadult or adult stages to detach. Lice found in the anaesthetic bath were counted, (4) the chalimii stages were recorded without differentiating the two sea lice species. The preadult stages of *L. salmonis* and the adult stages of *C. elongatus* are also difficult to differentiate. When the abundance of *C. elongatus* is high, the recorded numbers of mobile *L. salmonis* may be affected. Reported counts of *L. salmonis* were evaluated and if necessary reduced to adjust for the possibility of chalimii and adult male *C. elongatus* being recorded as *L. salmonis*, (5) finally, it was always necessary to count sea lice in the closed cages first, and to use cage water for the anaesthetic bath to prevent contamination.

A standard procedure for sampling fish and counting lice recommended from the Norwegian Seafood Federation (NSF) was employed (Norwegian Seafood Federation, 2013). Fish were collected as random as possible from the cage, using a crowding net. The fish were lifted with a dip net into a tank with sedation (Benzoac®, 2 ml·10 l^{-1} or Aqua-calm®, 0.5 g·10 l^{-1}) and sedated until swimming activity ceased, they lost vertical balance and the muscular reflexes were so reduced that they could be lifted, weighed and handled without danger of acute stress or physical injury. The sedation water was changed and the number of sea lice in the water recorded between each cage. After counting sea lice the fish were usually weighed and then released back into the cage or released into a separate tank with fresh, circulating water to let them regain normal swimming behaviour before we returned them to the cage. Fish sedated with Aqua-calm were always killed because it is prohibited to use methomidate to food producing animals.

2.4. Mortality and growth

Dead fish were collected and counted by the farm staff, recorded in farm databases and reported monthly together with lice counts and information on feed use. The survival rate (SR %) was calculated as:

$$SR\% = (n_2/n_1) \cdot 100$$

where n_1 = number of fish stocked in the cage at day 1, n_2 = number of fish recorded in the cage at the end of the project. The counting of fish through commercial counters in the hatchery or well boats is not accurate, and retrieval of dead fish from such large cages will not provide exact estimates of mortality. Therefore, there were divergences between different estimates of the number of fish in all cages. The most accurate figures were believed to be the numbers received from the hatchery when moving smolt to seawater, along with the slaughter reports, so these numbers were used when possible.

The weight is also difficult to monitor in groups of this size. Consequently, the data used were a combination of figures from the farm databases and individual weights recorded when counting sea lice and at all other samplings performed by the research team. When fish groups were harvested, accurate reports on number and weight distribution were collected from the slaughterhouse. Growth was calculated as Thermal Growth Coefficient (TGC), from the formula:

$$TGC = 1000 \cdot \left({w_2}^{1/3} - {w_1}^{1/3}\right)/(T \cdot t)$$

where w_2 is end weight and w_1 start weight, T is average temperature in °C and t is time in days.

2.5. Sea lice treatments and bioassays

Treatment for sea lice in open cages was performed by: (1) in-feed medication with emamectin benzoate (Slice®) or teflubenzurone (Releeze®), (2) bath treatment with pyrethroids (deltamethrin, AlphaMax®), (3) bath treatment with organophosfates (azametiphos, Salmosan®), (4) bath treatment with hydrogen peroxide, (5) mechanical removal of lice by use of high pressure water systems, (6) use of cleaner fish (goldsinny wrasse, *Ctenolabrus rupestris,* or lumpfish, *Cyclopterus lumpus*) or with combinations of these treatments. In some of the open cages, the nets were wrapped in plankton sheeting (sea lice skirts) to prevent an influx of sea lice larvae at the surface. Sea lice collected from salmon in open cages were tested with single- or multiple-dose bioassays in August 2011, November 2012 and August 2013 (Helgesen and Horsberg, 2013; Sevatdal and Horsberg, 2003; Westcott et al., 2008; Whyte et al., 2013). The choice of therapy, dosage and timing of treatments was coordinated with nearby farms and the participating well boats, in cooperation with the regional NSF coordinator.

2.6. Fish welfare

All fish that were handled were either sedated and handled carefully or killed by an overdose of anaesthesia or a sharp blow to the head followed by cutting off the gill arcs. Individuals accidentally injured during registration or with signs of severe illness, lesions or deformities were killed to avoid further suffering.

3. Results

3.1. Sea lice in closed and open cages

We performed 180 sea lice counts (3597 fish) in closed cages and 197 counts (3729 fish) in open cages (Tables 3–8). In the 6 closed cages stocked with smolts (Cages no. 1, 6, 17, 18, 19, 20), only 2 lice were found; one mobile *L. salmonis* in Cage no. 6, August 2013, and one adult *C. elongatus* in Cage no. 18, March 2015 (Fig. 3., panels c, e). In 5 closed cages stocked with post-smolts after transfer of salmon between closed cages at Site 1 (Cages no. 3, 8, 9, 12, 13), the recorded abundance was higher, with maximum total abundance from 0.05 to 0.32 (Table 2, Fig. 4). The first sea lice were found 15 to 66 days after stocking (Cage no. 3), and infestation persisted up to 253 days after this (Table 4). The abundance in these closed cages was gradually reduced without treatment. Only one chalimus stage of *L. salmonis* was found in any of the closed cages (Cage no. 13), 18 days after stocking the cage with post-smolts in August 2014 (Fig. 4, panel e, Table 7). For the majority of the closed cages between 70 and 100% of the lice counts showed no sea lice (Table 2). The exceptions were Cage no. 8 (42%) and Cage no. 12 (0%). Cage no. 12 was stocked with 33,194 post-smolt

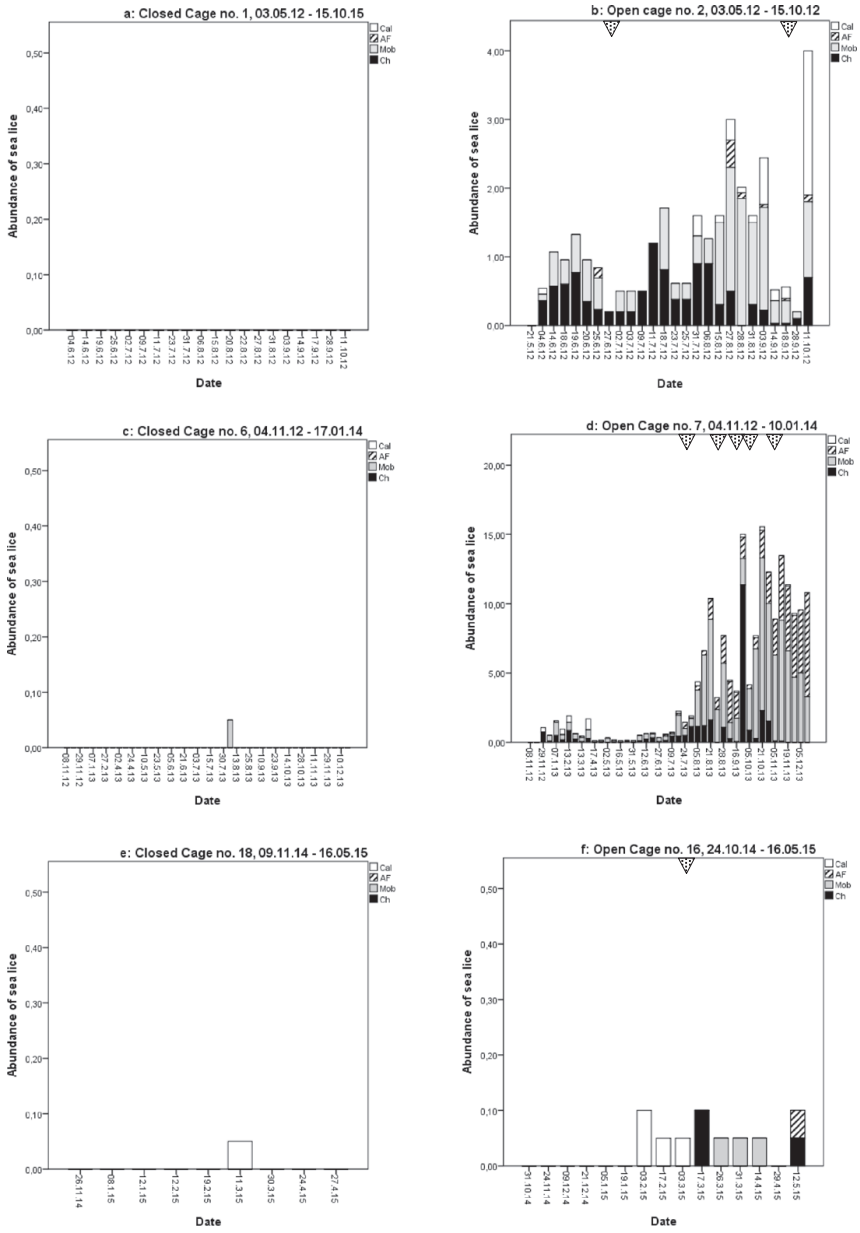

Fig. 3. The abundance of sea lice on salmon smolts in closed and open cages. Side by side panels show closed cages (left panels) and open reference cages (right panels). Panels a to d: Site 1, panel e: Site 4, Panel f: Site 3. Ch = chalimus, *L. salmonis* and *C. elongatus*, Mob = preadult *L. salmonis* and adult males, *L. salmonis*, AF = adult females, *L. salmonis*, Cal = adult *C. elongatus*. The first lice count after chemical treatment for sea lice is indicated by arrows in top of each panel. The first count after chemical treatment is indicated by arrows in the top of each panel. Cleaner fish (*Cyclopterus lumpus*) was also used in open Cage no. 16, panel f. Note the difference in scale in the panels.

(weight 740 g) in April 2014. The fish was retrieved from an open cage at site 3, treated for sea lice with a bath of hydrogen peroxide in the well boat during transport and then released into the closed cage. Sea lice were found in all the 13 lice counts (May to July), median abundance was 0.15 and most lice were classified as mobile *L. salmonis* (preadult females, preadult and adult males), with a few adult female *L. salmonis* and adult *C. elongatus*.

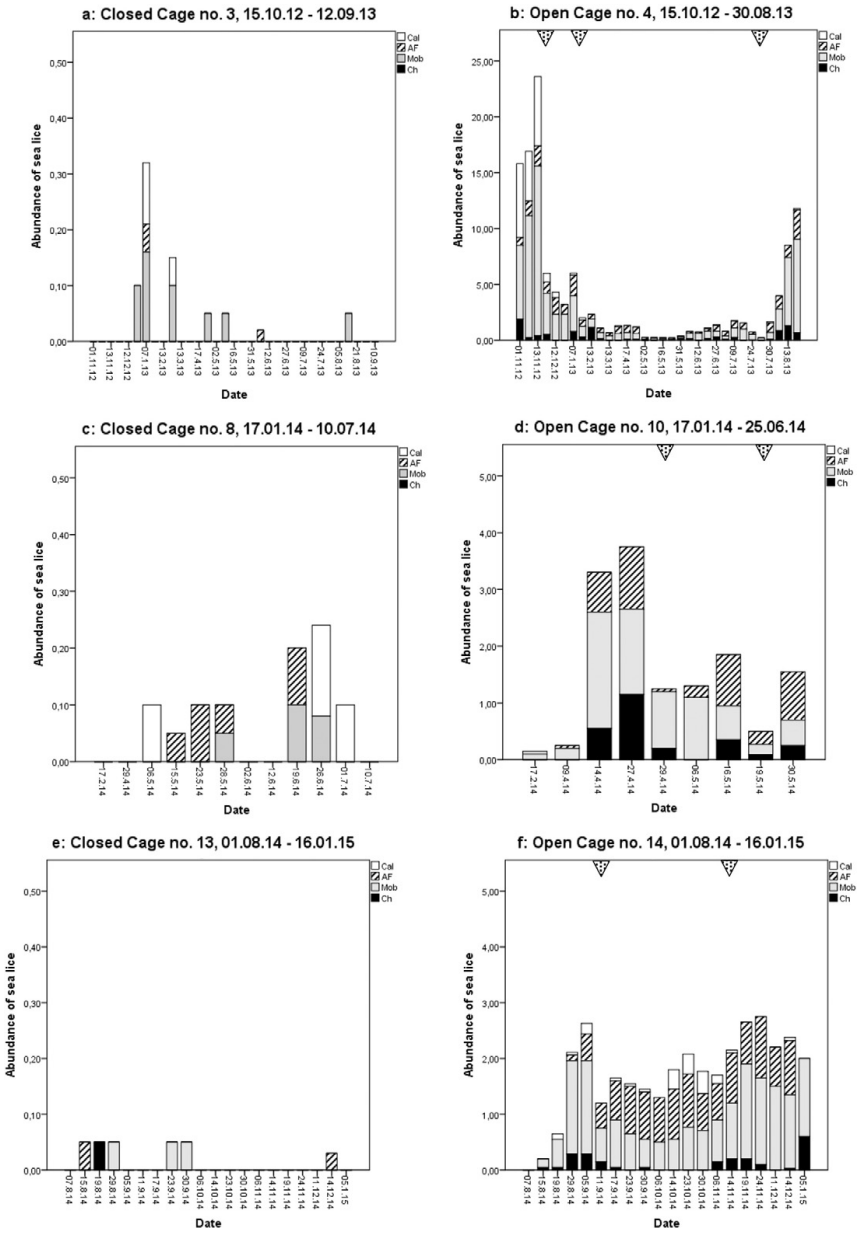

Fig. 4. The abundance of sea lice on salmon post-smolts in closed and open cages. Side by side panels show closed cages (left panels) and open reference cages (right panels). Cage no. 10, panel d: Site 3, all other cages: Site 1. Ch = chalimus, *L. salmonis* and *C. elongatus*, Mob = preadult *L. salmonis* and adult males, *L. salmonis*, AF = adult females, *L. salmonis*, Cal = adult *C. elongatus*. The first lice count after chemical treatment for sea lice is indicated by arrows in top of each panel. Cleaner fish (*Ctenolabrus rupestris*) and skirt was also used in open Cage no. 14, panel f. Note the difference in scale in the panels.

The open cages were infested with sea lice throughout most of the year, with highest abundance from July to November (Picture 2). Chalimii were recorded in most lice counts. The majority of lice were identified as *L. salmonis*, but shorter periods with increased abundance of adult *C. elongatus* were recorded. In the 4 open cages stocked with smolts (Cages no. 2, 7, 15, 16) the maximum abundance varied from 0.10 to 15 (Fig. 3, Table 2, 3,5,8). In the open cages stocked with post-smolts, maximum abundance of sea lice

varied from 0.24 to 23.6. The highest abundance in open cages was recorded in Cage no. 4 (23.60) (Fig. 4., panel b), and in Cage no. 7 (15.00) (Fig. 3., panel d). For the majority of lice counts in open cages between 64 and 100% of the lice counts showed abundance >0 (Table 2). The exceptions were Cage no. 15 (43%) and Cage no. 16 (53%).

Fish in closed cages were never treated for lice. Different measures to prevent or treat against sea lice were implemented in all open cages, see *2.5 Methods*. In nine open cages, a total of 21 chemical treatments were used against sea lice; the mean number of treatments was 2.3, ranging from 0 to 5 (Table 2). Sea lice collected from Site 1 in November 2012 and August 2013 showed resistance to the chemoterapeutants commonly used in the region (azametiphos, deltamethrin, emamectin benzoate). As a result of this, 4 out of 6 open cages with market sized salmon (Cages no. 7, 10, 11, 14) were harvested earlier than scheduled to eliminate the local population of resistant sea lice after repeated and partly unsuccessful chemical treatments.

3.2. Survival rate and growth

In closed cages, the lowest survival rate (75.6%) was recorded in Cage no. 6 (Table 1). The majority of mortalities in this group were identified as winter ulcers with isolation of the bacteria *Moritella (Aliivibrio) viscosa* and *Tenacibaculum* sp., and mortality peaked from January 2013 to April 2013. In the other closed cages, survival ranged between 92.1 and 99.1%.

In the open cages, the lowest survival rate was recorded in Cage no. 7 (71.1%) and in Cage no. 2 (78.3%). In both cases, the mortality was caused by toxic side effects of bath treatments (azametiphos or deltamethrine) against sea lice. In the other open cages, the survival rates were between 94.0 and 99.2%.

The lowest growth rate was recorded in the first closed cage with 80,000 smolt (TGC = 2.2), in the open Cage no. 10 with 22,775 post-smolt (TGC = 2.2) and in the closed Cage no. 6 with 80,000 smolt (TGC = 2.4). In the other closed cages, TGC ranged between 2.6 and 3.6 and in the open cages between 2.6 and 3.8.

Table 2
Summary of sea lice counts in closed and open cages, May 2012 to May 2015. Number of sea lice counts in each cage, % of sea lice counts with total abundance = 0 and % of counts with total abundance > 0, maximum abundance recorded in each cage and sum of chemical treatments. Other measures against sea lice as wrasse or skirts are not included.

Cage type	Fish size	Site no.	Cage no.	No. counts	% Ab. = 0	% Ab. > 0	Max Ab.	Chemical treatments
Closed	Smolt	1	1	20	100	0	0.00	0
Closed	Smolt	1	6	47	98	2	0.05	0
Closed	Smolt	4	17	7	100	0	0.00	0
Closed	Smolt	4	18	9	89	11	0.05	0
Closed	Smolt	4	19	7	100	0	0.00	0
Closed	Smolt	4	20	6	100	0	0.00	0
Closed	Post-smolt	1	3	34	79	21	0.32	0
Closed	Post-smolt	1	8	12	42	58	0.24	0
Closed	Post-smolt	1	9	5	80	20	0.06	0
Closed	Post-smolt	1	12	13	0	100	0.20	0
Closed	Post-smolt	1	13	20	70	30	0.05	0
Open	Smolt	1	2	26	4	96	4.00	2
Open	Smolt	1	7	44	5	95	15.00	5
Open	Smolt	3	15	14	57	43	0.20	1
Open	Smolt	3	16	15	47	53	0.10	1
Open	Post-smolt	1	4	33	0	100	23.60	3
Open	Post-smolt	2	5	25	12	88	13.25	5
Open	Post-smolt	3	10	9	0	100	3.75	2
Open	Post-smolt	1	11	11	36	64	0.24	0
Open	Post-smolt	1	14	20	5	95	2.75	2

3.3. Water flow and quality

Temperatures in the cages depended on cage type, season and site. In the open cages, temperatures fluctuated with depth, in closed cages, the temperatures were always homogenous from the surface to the bottom of the tarpaulin. The temperature in closed cages at Site 1 was similar to the open cages from mid-September to mid-May, but 1–2 °C lower during the summer. At Site 4, there was a thermo- and halocline during winter, with 0.5 °C higher average temperature in closed cages compared to the open reference cages at Site 3 (Table 8). In all open and closed cages, recorded salinity was >30 ppt.

The total exchange rate of the water volume in the closed cages was reduced from 250 min (in 2012 and 2013) to 120 min (in 2014 and 2015). Recorded levels of CO_2 were <15 mg $CO_2 \cdot l^{-1}$ except in closed Cage no. 1 (August to October 2012) and in Cage no. 6 (December 2013 to January 2014).

4. Discussion

The observed abundance of sea lice in all closed cages with smolts was close to zero (Fig. 3, Tables 2, 3, 5, 8), and this was repeated in 6 closed cages (Cages no. 1, 6, 17, 18, 19, 20) throughout three different production periods (Tables 3, 5, 8), at two different sites (Sites 1 and 4) and with reference groups in 4 open cages (Cages no. 2, 7, 15, 16) at Site 1 and Site 3. The presence of infective sea lice larvae in the surface water around the closed cages (Cages no. 1 and 6) at Site 1 was confirmed by permanent sea lice infestation and periods with high abundance in reference groups in the open cages at the same site (Fig. 3, Tables 2, 3, 5). At Site 4 there were no open cages as the permit for this site was for closed cages only. The reference cages (Cages no. 15 and 16) were located at Site 3, both with a low abundance (0.05 to 0.20) of sea lice (Tables 2 and 8). These cages were also stocked with cleaner fish and received one chemical treatment. The inlet water of the closed cages was pumped from 25 m depth and was not filtrated or otherwise treated to remove sea lice, and on the surface, the cages were covered only by a net to keep out predatory birds. The closed cages were modelled to withstand waves of 0.75 m and located at two sheltered sites. Still, during winter seasons all sites were exposed to violent storms with the wind occasionally exceeding 30 $m \cdot sek^{-1}$, and the cages were occasionally covered in spray. Thus, it is likely that the sole 2 sea lice found in 2 of the 6 closed cages stocked with smolts (Cages no. 6 and 18, Fig. 3, Tables 5 and 8) were either introduced through the inlet water, by contamination from the surface, or from the equipment used for sea lice counting. It is not likely that lice were introduced into the cages during stocking with smolts. Earlier studies have showed reduced abundance of sea lice when the contact between farmed salmon and surface water was restricted (Grøntvedt and Kristoffersen, 2015; Hevrøy et al., 2003; Korsøen et al., 2012; Stien et al., 2016). The results of our study show that floating enclosures with a fixed intake depth below the vertical dispersion range of infective copepodites provide sufficient protection against sea lice.

The abundance of sea lice was higher in the 5 closed cages stocked with post smolts than in the closed cages stocked with smolt. However, the corresponding open control cages showed much higher lice counts and were continuously treated to keep the abundance in accordance with the legislation. The challenge with use of different sea sites and possibilities of surface contamination also applied to the post-smolt cages. During the project period, there were no serious technical accidents leading to increased risk of sea lice infestation in the closed cages. Still, sea lice may be introduced by management practices such as stocking cages with infested fish (Cage no. 12) or exposure to surface water during management procedures such as transfer of fish between cages (Cages no. 3, 8, 9, 13). As the post-smolt grew from 100 g to 5000 g in the closed cages, it was necessary to split the biomass at least once during the production cycle. During this procedure, the salmon from

the closed cages were loaded into well boats (Picture 3) with continuous water exchange from the surface water outside the cages (2 m depth). The water volumes from the well boats were also unloaded into the new closed cages together with the fish, as the well boats had no technology available to filtrate the water intake or discharge. Thus, these operations exposed the salmon to surface water with a possible high concentration of infective sea lice. The low sea lice abundance in the similar cages stocked with smolts, makes it likely that the infestations in the closed post-smolt cages are due to stocking from an open cage or from the limited exposure to surface water during the well boat procedures.

When the infestation was established, a steady supply of chalimii should have been detected if the environment inside the closed cages allowed sea lice to develop into the infective stage and complete its life cycle within the cages. However, only one chalimus was found in (Cage no. 13, 18 days after stocking), indicating that a self-sustaining infection had not been developed in any of the closed cages with post-smolt. The abundance remained low, and it was never necessary to treat against sea lice in any of the closed cages. Adult sea lice are able to live at least 6 months on their hosts (Hevrøy et al., 2003), and if the infestation occurred during stocking, this could explain the duration of moderate sea lice abundance in the closed cages, even with the absence of chalimii. In one closed cage (Cage no. 12) we found a low and continuous abundance of preadult and adult sea lice, but also here chalimii were absent. This group of fish was exposed in an open cage for a longer period (from November 2013 to April 2014) before they were moved to the closed cage. The prevalence may have been higher in this group compared to the groups moved between closed cages, and this could explain why preadult and adult sea lice were detected for a much longer period in this cage. In the closed cages, the water exchange rate ranged from 120 to 250 min. The nauplii of *L. salmonis* hatch from released egg-strings and moult into infective copepodites after 50 degree-days (Boxaspen and Naess, 2000; Stien et al., 2005), while the third and infective planktonic copepodite stage lasts approximately another 100 degree-days. For *C. elongatus* it takes 36 to 41 degree-days to develop from eggs to infective copepodites (Pike et al., 2006). The magnitude of water exchange in the closed cages would most likely make it difficult for lice to hatch and develop into the infective stage before they were flushed out of the system, thus terminating the life cycle within the cage. The lack of sea lice reproduction inside the cages may also have been caused by mate limitation. Sea lice were counted on juvenile salmon migrating past salmon farms in British Columbia, Canada (Krkošek et al., 2012). The study showed that mate limitation occurs for salmon lice and that there is a limited scope for increase in parasite survival at low abundances. Regardless of the mechanisms behind the low reproductive success, it appears that the environment inside the closed cages is able to cope with at least small infestations of lice.

Future research should include more specific modelling of the vertical dispersion range of copepodites on the sea sites and studies of the effect of different levels of sea lice infestation in the closed cages.

5. Conclusions

Farming of Atlantic salmon in closed, floating cages with water intake at 25 m offers an effective protection against sea lice (*L. salmonis* and *C. elongatus*). When sea lice were introduced into closed cages, no signs of reproduction or continuous infection were recorded. Preliminary production data indicates that production in closed cages could give acceptable survival and growth rates compared to traditional open cages. Further studies on technical stability, water quality, fish welfare and biological and economical results are necessary to evaluate the sustainability of this new cage technology.

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II

Performance of post-smolt Atlantic salmon in closed confinement systems: Growth, mortality and fish welfare

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Abstract

The most controversial environmental issues in Norwegian salmon farming are the negative effects of salmon lice (*Lepeoptheirus salmonis*), genetic interogression of farmed salmon in wild populations, nutrient load and the emission of potentially toxic waste to coastal waters. If these challenges are left unresolved, the future growth of salmon farming will be restricted or even reversed as a result of the new 'traffic light' system implemented by Norwegian authorities from 2018. Moving production from marine net-pens to land-based facilities, offshore farming or closed containment systems (CCS) are suggested as possible ways to solve these problems. The main aim of this study is to describe growth rates, mortality rates and mortality causes in commercial scale CCS, and to compare growth and mortality with data from net-pen farming. We tested 18 CCS with off-season smolt (S0), 5 CCS with one-year smolt (S1) and two net-pens with S0. No salmon lice were found in any of the CCS. Post-smolt salmon in CCS showed high growth rates and equal to or lower mortality rates compared to data from commercial farming in net-pens. The mean (SD) TGC for all 23 CCS was 3.04 (0.37), with little difference between one-year smolt (S1) and off-season smolt (S0). Total cumulative mortality three months after sea transfer (CM_{3mo}) was 2.6%, while cumulated mortality after the total trial period (CM_{total}) was 3.6%. The two main mortality causes were 'Ulcers and fin rot' and 'Failed smolt' accounting for 35.3% and 18.7% of the total mortality, respectively. Increased growth rates in CCS could be explained by higher water velocities and more aerobic training. Water flow, oxygen saturation and other water quality parameters were within safe limits for fish

health and welfare. Observations of arameters such as water quality and water velocity are also discussed in a fish welfare context.

Keywords: *Salmo salar*; Closed containment systems; Mortality, Growth rates, Fish welfare

Introduction

The growth of salmon farming in Norway has been arrested due to increasing awareness of its negative environmental impacts; special attention has been given to the negative effects on wild salmonid populations caused by salmon lice (*Lepeophteirus salmonis*). The emergence and rapid spread of drug-resistant lice have forced farms to abandon chemical treatments and to develop non-medicinal treatments or alternative farming strategies (Aaen et al., 2015; Overton et al., 2018; Helgesen and Jansen, 2019). Consequently, Norwegian authorities established a so-called 'traffic light system' where estimates of sealice-induced mortality on wild, migrating salmon smolt are used to regulate the growth of salmon farming (Norwegian Ministry of trade, industry and fisheries, 2017a). Negative effects on wild salmon populations caused by the spread of diseases and escaped fish (Naylor et al., 2005; Garseth et al., 2013) and the potential negative effects of nutrient overloading in coastal areas (Braaten, 2007) are also important issues to solve.

The development and implementation of new farming technologies could mitigate these negative environmental impacts. In closed containment systems (CCS), intake water is pumped from deeper water layers, making it possible to avoid all infective salmon lice copepodites (Nilsen et al., 2017a). Fish escape from net-pens, mostly because of broken nets, caused by rough weather conditions or as a sequel tooperations such as treatments against salmon lice (Jackson et al., 2015; Anonymous, 2018a). The risk of escaped fish could possibly be reduced by locating CCS at sheltered sea sites. In addition, with CCS it is possible to collect and reuse settleable particles from faeces and surplus feed.

Knowledge about the biological results from production in larger closed containment systems is surprisingly scarce (Calabrese, 2017; Tveterås and Misund, 2019). A thorough review of biological requirements for post-smolt Atlantic salmon in CCS was published by Thorarensen and Farrell (2011), and a Norwegian report assessing the potential of CCS technology is available (Rosten et al., 2011). In recent years, two doctoral theses (Calabrese, 2017; Sveen,

2018) and a few studies of large-scale CCS (Summerfelt et al., 2016; Nilsen et al., 2017a,b; Balseiro et al., 2018; Karlsen et al., 2018) have been published. Several experimental studies of stocking density and specific water consumption with special reference to implementation in CCS or in RAS systems have been reported (Sveen et al., 2016; Calabrese, 2017; Gorle et al., 2018; Sveen et al., 2018). The effect of swimming exercise has been described by Nilsen et al. (2018), exercise at different salinity levels by Ytrestøyl et al. (2017) and at different temperatures by Hvas et al. (2017). A number of studies conducted in the late 1980s and early 1990s describing ongrowing of post-smolt salmon in land-based, flow-through tanks supplied with oxygen-enriched sea water are available, emphasising growth rate, feed utilisation and mortality (Forsberg, 1995). In addition, a pilot study on the ongrowing of post-smolt salmon in closed, small tarpaulin covered cages (CCS) was performed in Southwestern Norway (Skaar and Bodvin, 1993).

To the best of our knowledge, there are few published studies on fish performance and rearing conditions in commercial scale salmon farming in CCS. The description and validation of sound biological limits for production capacity are necessary to avoid reduced biosecurity or compromised fish welfare (Ashley, 2006; Thorarensen and Farrell, 2011). Detailed data are also needed for the purpose of establishing more precise socio-economic models. The present study's main aim was to describe growth rates, mortality rates and mortality causes during a pilot study of commercial scale production of Atlantic salmon in CCS. Other relevant health and welfare data are also included.

Materials and Methods

2.1 CCS technology and trial groups

During the period from October 2014 to May 2017, three different sea sites in the southern part of Nordland county, Norway, were used (Figure 1)

- Site 1 (site no. 33837): four CCS, tarpaulin semi-globes of 2870 m^3 (ø = 22 m, d = 14 m) with two inlets as described in Nilsen et al. (2017a) (Figure 2).
- Site 2 (site no. 10425): two net-pens, circular with approximate volume 30 000 m^3 (ø = 51 m, d = ca 30 m).
- Site 3 (site no. 35737): 10 CCS tarpaulin semi-globes of 6000 m^3 (ø = 28 m, d =17 m), with four inlets (Figures 3 and 4).

We performed trials with one-year smolt (S1) and off-season smolt (S0), with 23 CCS and two net-pens. Four trial groups were defined for graphic presentations:

1. CCS with S0 at site 1 (n=12)
2. Net-pens with S0 at site 2 (n=2)
3. CCS with S1 at site 3 (n=5)
4. CCS with S0 at site 3 (n=6).

In all CCS, the impermeable tarpaulin bags were filled with water pumped (5.5 kW, Xylem Norway AS) from 20-25 m depth (Nilsen et al., 2017a), and drained through one central outlet. Sedimentable particles and dead fish were separated from the water flow in the outlet and pumped in separate tubes to the surface. The CCS were circular with open-ended inlets located at 1-1.5 m depth, creating a circular, primary horizontal current. Each cage was supplied with external light mounted on the floating ring supporting the tarpaulin bags (LED 2x50W 230V IP65, Etman Distribusjon AS, Egersund, Norway).

2.2 Water quality and water velocity

Oxygen to the CCS was supplied by a diffusor net (AkvaDesign AS); oxygen and temperature were logged at 10-minute intervals at 2 m depth (system: FDO 700 IQ SW, WTW/Xylem). Mean oxygen saturation was regulated to 80-95% in all CCS. Water quality parameters such as pH, temperature, dissolved oxygen and salinity were also measured with SmarTroll MP handheld sensor (Tormatic AS, Norway). Carbon dioxide was measured with OxyGuard CO_2 portable meter (OxyGuard AS, Denmark). Water samples for laboratory analysis of pH (NS-EN ISO 10523), total ammonia nitrogen (TAN, NS-EN ISO 14911) and total suspended solids (TSS, NS-EN 872) were collected at different depths in the cages, in the water column outside the cages and in the inlets with a Ruttner type water sampler (Fybikon AS, Norway), and stored at +4 ºC in sterile plastic bottles for chemical water analysis until delivery to the laboratory (Kystlab Prebio, Brønnøysund, Norway). Cross-sectional water quality profiles were measured in CCS no. 8 and no. 9 four times during the period from January to April 2016. The water velocity in CCS was measured in December 2016 (site 1) and May 2016 to May 2017 (site 3). Water velocity at site 2 was measured over a period of 30 days in October 2011 by MarinKonsulent. Water velocity was measured with SD6000 current sensors (Nortek AS) at 1 to 10 minute intervals, downloaded and analysed with SD6000 software (number of measurements, mean, standard deviation, significant minimum and maximum velocities.

Figure 1

Figure 2

Figure 3

Figure 4

2.3 Fish and rearing conditions

The Atlantic salmon smolt (*Salmo salar*) in the study population (Table 1 and Supplementary data 1) were delivered from three different hatcheries with sea transfer between May and June as one-year smolt (S1) or between October and December as off-season smolt (S0). When cages are referred to by numbers, these are the chronological numbers assigned in Supplementary data 1. The fish were of AquaGen or Salmobreed strain, selected for IPNV resistance by use of quantitative trait loci (QTL) methods (Anonymous, 2013). Off-season smolt were smoltified with an artificial light regime, one-year smolt with a natural photoperiod. Smoltification quality was measured before sea transfer with a combination of morphological evaluation and one or several of the following laboratory test procedures: determination of plasma chloride after 48 h exposure to sea water, measurement of levels of gill ATP-ase (Pharmaq analytic AS) or Smolt-Timer (Patogen AS). Fish in seven of the CCS (1, 2, 3, 4, 15, 21, 24) were reared for two to four weeks in brackish water (14-20 ppm) before sea transfer; all the other groups were reared in freshwater (salinity ≤ 1.5 ppm) until sea transfer. All fish were of a selected vaccinated with commercial oil-based vaccines against *Aeromonas salmonicida*, *Vibrio salmonicida*, *Vibrio anguillarum* serotypes O1 and O2a, *Moritella viscosa* and infectious pancreas necrosis virus (IPNV). The smolt groups were transported to the sea sites in well boats and monitored until they were transferred as post-smolt to new cages or mean weight reached 1000 g. All fish were fed until satiation with commercial pelleted feed (Skretting AS, Biomar AS), at sites 1 and 3 with automatic pneumatic feeding systems (AkvaGroup AS), and at site 2 with Betten feed automats.

Unfortunately, problems with drug-resistant salmon lice escalated in this region during the period from 2013 to 2016. Increased treatment frequencies and sometimes also forced harvesting was necessary to keep down the salmon lice abundance at sea sites with net-pens. These measures against lice had a heavy impact on growth rates and mortality rates at the most affected sites. Thus, it was difficult to establish comparable, long-term growth studies involving CCS and commercial scale net-pens, in addition, the research sites were only licensed for CCS. All trials were performed in a commercial or semi-commercial setting, with standard

operational procedures applied regarding feed and feeding, transport and handling of live fish, health surveillance and humane treatment of individual fish during sea lice counts, weighing procedures or when culling fish. Permission from the Norwegian Animal Research Authority was not required.

Table 1

2.4 Growth and mortality rates

Size of individual fish was recorded in weight (W) as round body weight in g (±1g), length (L) as fork length (±0.5 cm), and condition factor: $CF=100\cdot(W/L^3)$.

Specific growth rate (SGR) was calculated as (Houde and Scheckter, 1981):

$$SGR=100\cdot(\ln(W_1)-\ln(W_0))/(t_1-t_0)$$

where W_1 and W_0 are weights on days t_1 and t_0, respectively.

Thermal growth coefficient (TGC) was calculated as (Alanärä et al., 1994):

$$TGC=1000\cdot(W_1^{1/3}-W_0^{1/3})/(T\cdot t)$$

where T is temperature in °C and t is time in days.

Specific feeding rate was calculated as:

$$SFR=(\text{feed/biomass})\cdot 100$$

where feed is weekly mean kg feed/cage/day and biomass is average biomass/cage/day in the same week.

Feed conversion rate was calculated as:

$$FCR=\text{total feed use (kg)/total increase in biomass (kg)}$$

Start weight (W_0) was determined by the smolt documentation from the hatcheries, verified with weight controls during the time of sea transfer. End weight (W_1) was determined by a combination of output from the production database (FishTalk, AkvaGroup AS) and weight controls. In total, 73 control samples with a total of 13,527 fish were used for weight adjustments at the three sites. Growth and mortality rates in each cage were calculated from the total production data (number of fish, mean weight at start and end, total feed consumption, total time period and mean temperature). In addition, weekly data were collected from each cage: number of fish, stocking weight, density, specific feeding rate, SGR, TGC, weekly mortality count, weekly mortality rate and the number of fish assigned to each of the defined mortality causes. Condition factors at sea transfer and trial conclusions were compared with the SWIM 1.0 welfare scoring suggested by Stien et al. (2013).

From 2014 to 2015, a cohort study of two CCS and two net-pens was performed to evaluate sea lice counts, mortality and growth rates. Smolt of similar origin and size were stocked into CCS nos. 2 and 4 and net-pens nos. 5 and 6. Smolt in net-pens were transferred directly from fresh water, smolt in CCS first to brackish water (described in *2.3 Fish and rearing conditions*). Production data from cages nos. 1 to 6 was also reported in the publication on sea lice in CCS (Nilsen et al., 2017a). During the revision of data from all the cages in this study, minor adjustments of W_1, T and t were performed for all six cages, leading to a small increase in TGC for cages nos. 1 to 5, but no changes in CM_{total}.

The number stocked in each cage (n_0) was given by the figures from the hatcheries, based on their records from vaccination, from which was subtracted the mortality between vaccination and sea transfer. For some cages the stocking numbers were rounded to the closest 1000. Cumulative mortality rates for the first three months after sea transfer (CM_{3mo}) and the total time period (CM_{total}) were calculated as the proportion of mortalities during the time period compared with n_0. Weekly mortality rates were reported as $(n_{week}/n_{risk})\cdot 100$, where n_{week}=weekly mortality count and n_{risk}=number of fish at risk at the start of the week. The final numbers in each unit (n_1) were calculated by subtracting CM_{total} from n_0. Cumulative mortality rates were compared to other studies from net-pens or CCS, and a welfare score based on CM_{3mo} was suggested.

Dead fish were collected daily. Injured or weak fish were netted, killed and recorded as culled. Dead or killed fish were inspected and cause-specific mortality was assigned as

1. ‘Culled’: Lethargic or injured fish with erratic and slow swimming, often close to the surface. These were collected, euthanized and recorded as culled
2. ‘Decomposed’: Rotten, not possible to evaluate the possible cause of death.
3. ‘Cachectic’: Emaciated fish, often with darker colour.
4. ‘Failed smolt’: Parr, discoloured or undersized, precocious males.
5. ‘Ulcers and fin rot’: Serious loss of scales and petechial haemorrhages, active and severe fin rot (grade 3), severe skin ulcerations where muscle tissue is exposed.
6. ‘Trauma’: Mortality immediately connected to procedures (like transport, grading or netting) where fish have been killed or severly injured
7. ‘Others’: Open category – whatever comes up as less important epidemic mortality and could be caused by parasites, bacteria or virus. Fish without any

specific signs of injury or disease (unknown cause of death).

'Culled' and 'Decomposed' were not exclusive mortality categories, but necessary to identify as part of the welfare management at the sites. The number of fish classified as dead due to predators, environment/water quality or by infectious diseases apart from ulcers and fin rot were low in all cages. These mortality causes were grouped together as other or unknown causes. The farms reported mortality as weekly numbers of fish assigned to each mortality group, except for cages 20-25 at site 2. In these CCS the authors performed weekly mortality classification during 2017, and the mortality in 2016 was not classified. Classification of mortality causes in all cages was verified by monthly visits of fish health professionals. Kidney and gill samples from two trial periods at site 1 were sampled for histological examination, according to standard procedures from the Norwegian Veterinary Institute.

2.5 Sea lice

Sea lice (*Lepeophteirus salmonis* and *Caligus elongatus*) were monitored as described in Nilsen et al. (2017a), following the Norwegian regulation on salmon lice in aquaculture (FOR-2012-12-05-1140). Data are available at the open access platform https://www.barentswatch.no. In CCS, counting of sea lice was performed less frequently during winter, because of the negative impact netting and counting at low temperatures has on fish welfare. To compensate for this, culled fish were examined for lice and sea lice were counted during weight samples. Counts of larger numbers of fish during the final weight samples were also important to reduce the probability of false negatives.

2.6 Statistical analysis

All production data were recorded daily at farm level and entered into the FishTalk database. Data were collected from each unit on a weekly basis: stocking weight (g), stocking number, stocking density (kg/m^3), total mortality count and cause-specific mortality count, feed use (kg), SFR, SGR, TGC and FCR. The total production data were also summarised for each cage unit, as described above. The weekly data and the total production data were transferred to Excel and to IBM SPSS 25 statistical package (IBM Corporation, NY, US). In the cohort trial in 2014-2015, sampled fish were measured individually (weight, length) and a mixed model (ML) linear regression method with cage type as fixed factor and cage as random factor was used to compare the results from net-pens and CCS. Water quality parameters from a cross-sectional study at site 1 in 2016 were first analysed with a mixed linear regression model (ML) with horizontal

distance as fixed factor and depth/cage as random factors and then with depth as fixed factor and horizontal distance/cage as random factors. The relationship between weekly values of SGR and water temperature and between SGR and TGC was first evaluated by calculating Pearson and Spearman's correlation coefficients and then by using a linear regression model.

Results

3.1 Cohort trial, 2014-2015

The cohort trial compared two CCS (nos. 2 and 4) with two net-pens (nos. 5 and 6). Reported weight at sea transfer was 121 g in CCS, 110 g in net-pens. Mean (SD) sampled weight after sea transfer (Table 2) was 112 (21) g in CCS, 106 (22) g in net-pens, with no significant difference in sampled weight or length, but significantly higher CF ($p<0.001$) in CCS. At the end of the trial, weight in CCS was significantly higher than in the net-pens (difference of 102 g, $p=0.001$), condition factor was still higher in CCS (difference of 0.13, $p>0.001$), and no significant difference in body length between the groups. Data on growth is presented in Tables 2 and 3 (sampled data) and Figure 5. Accumulated mortality was low (Table 4) with small differences between cages and groups. Cause-specific mortality rates (Table 5) were dominated by 'Other' (both groups) and 'Ulcers and fin rot' (most in CCS). Temperature and salinity profiles showed a thermo- and halocline between 3 and 10 m depth at site 1 (CCS-site) (Figure 6). Water quality measurements are summarised in Table 6. Dissolved oxygen (DO%) ranged from 74-86% in CCS, 85-110% in net-pens and 82-109% in the sea outside the CCS at site 1. In CCS, pH decreased and concentration of carbon dioxide increased slightly over time, with highest levels of CO_2 of around 2 mg/L recorded at the last sample in April 2015. Carbon dioxide in CCS ranged between 1 and 3 mg/L , pH in CCS between 7.52 and 8.09 and pH in the sea outside the CCS between 7.97 and 8.33. Water flow in CCS at site 1 was estimated at 12-14 m^3/min, corresponding to a retention time of 220 minutes. Maximum biomass in the two CCS was 46 and 50,000 kg, with density of 16 to 17.4 kg/m^3. With final feeding rates of around 0.8%, calculated maximum feed load in CCS was 21 g/m^3/day and minimum specific water consumption 0.26 L/kg. Water velocities were not measured in this trial, but were instead measured in two identical CCS at the same site one year later, with mean velocities between 13.8 and 22.0 cm/s. At site 2, water velocities at 5 m depth during October 2011 showed a mean water velocity of 3.7 cm/s, with significant minimum 2.0 and maximum 5.7 cm/s (data from MarinKonsulent).

Table 2
Table 3
Table 4
Table 5
Table 6
Figure 5
Figure 6

3.2 Growth rates, temperatures and FCR

Growth data from all 25 individual cages are reported in Supplementary data 1. In 23 CCS, the post-smolt took an average of 159 days at 8.0 °C to grow from 104 to 637 g, with a maximum density of 22.4 kg/m^3, a total TGC of 3.04 and FCR of 1.06 (Table 7). Seasonal fluctuations of temperatures at the three sites are shown in Figure 7.

Weekly data (from the production data base) on TGC and mean mortality rates are shown in Table 8 and Figure 8 (and in Supplementary data 2, Weekly data). Weekly specific feeding rates (SFR) and water temperatures were plotted against week after sea transfer (data not shown). Mean (SD) weekly SFR for S1 was 1.19 (0.42) with a mean (SD) temperature of 9.6 (2.4) °C. SFR for S0 was 1.15 (0.38) with a water temperature of 7.6 (1.4) °C. A significant linear correlation ($p<0.01$) between SFR and temperature existed in both seasonal groups, but with best fit for S0 cages. The precision of feeding and feed-monitoring in S1 cages was probably negatively affected by a peak in mortality during the first four weeks. Mean FCR from these five CCS was 0.94, we evaluated this as an unlikely positive outcome and FCR from the five CCS with S1 was thus excluded. Sampled weight (g) and condition factor (CF) at sea transfer and/or end of trial period for some of the cages are shown in Table 9.

Table 7
Table 8
Table 9
Figure 7
Figure 8

3.3 Mortality rates and mortality causes

CM $_{3mo}$ for all salmon in CCS was 2.6%, CM_{total} (159 days) was 3.6% (shown in the 'Total

mortality' column in Table 4). The mortality in CCS was higher in cages with one-year smolt (S1) than in cages with off-season smolt (S0). More than 72% of mortality occurred during the first three months after sea transfer (representing 57% of the total trial period). The 5 cages with lowest mortality rates (mean n_0=97,644, mean CM_{total}=0.91%) represented 4.3% of the total mortality, while the 5 cages with highest mortality (mean n_0=143,880, mean CM_{total}=7.3%) represented 53.2%. In Table 4, mortality rates at cage level (columns under 'Cages') are also summarised as mean, SE, median, minimum and maximum values. CM_{total} in the 23 CCS ranged between 0.7 and 10.9%, with median 2.1% and interquartile range (IQR) of 1.2-4.2%. The mean values of cumulated mortality at cage level were close to the total CM_{3mo} and CM_{total}. The median levels were lower than the means, especially for the first three months, caused by a skewed mortality pattern, with low mortality in the majority of the cages.

The three most frequent mortality categories in all cages were 'Ulcers and fin rot' at 35.3%, 'Other' at 29.1% and 'Failed smolt' at 18.7% (Table 5). 'Ulcers and fin rot' and 'Other' were recorded in all 25 cages, 'Failed smolt' in only 5 cages, all of them CCS with S1. The proportion of dead fish that were too decomposed to specify was 7.0%. In CCS with problems with 'Failed smolt', 'Ulcers and fin rot' was also an important cause of death, and 'Culling' was a necessity to meet husbandry standards of fish welfare. Figure 9 shows a graphic presentation of the causes of death in the 23 CCS, grouped as S1 and S0.

Figure 9

3.4 Specific water consumption, feed load, fish density and water velocity

The minimum specific water consumption at site 1 was between 0.22 and 0.29 L/kg/min, at site 3 between 0.20 and 0.50 L/kg/min. The maximum feed load at site 1 was between 19 and 26 g/m^3, at site 3 between 11 and 30 g/m^3. Density at sea transfer in the CCS (both sites) ranged between 1.9 and 4.2 kg/m^3, with maximum densities at the end of trials between 10 and 22.4 kg/m^3. Water velocity showed variation primarily along the horizontal axis, with less variation between different depths. In cages nos. 12 and 13, horizontal water velocity slowed down towards the cage centre, while in the larger cage, no. 20, the highest velocities were recorded at cage centre. Water flow during measurement of velocity in cages nos. 12 and 13 was estimated at 13 m^3/min, in cage no. 20 around 24 m^3/min, with a retention time of 220 and 250 minutes, respectively. Mean velocity (SD) across all measuring points at site 1 was 16.9 (2.9) cm/s

(ranging from 12.8 to 22.9 cm/s), while at site 3 mean (SD) velocity was 19.7 (1.9) cm/s (ranging from 15.6 to 22.6 cm/s). Measured water velocity in four different CCS at site 3 during 2016 showed larger variation of both water flow and water velocities (13.3 to 31.1 cm/s, data not shown). Water velocities at site 2 (net-pen site) were measured in 2011, as described in *3.2 Cohort trial, 2014-2015,* with a mean (significant min and max) value of 3.7 (2.0-5.7) cm/s. For sea sites in this region, mean water velocities at 10-15 m depth are usually <10 cm/s (Linda Hagen, AquaKompetanse AS, pers.com).

3.5 Water quality

Temperature (°C), oxygen saturation (DO%), salinity (ppt), pH and CO_2 measured by the authors are reported in Table 6. The values of pH are also converted to mg/L of CO_2, based on Nilsen et al. (2017b) and a calibration of pH vs. CO_2 was performed by the authors and Sveinung Fivelstad (Western Norway University College) at site 3 in 2017 (unpublished data). The oxygen saturation in the ocean outside the cages ranged from 80.7-130.9%. Inside the cages, variation in oxygen saturation was within safe limits for Atlantic salmon, with a mean value of 105% in net-pens and 81-86% in CCS. Median pH in CCS at the two sites was 7.8 and 7.5; pH in net-pens was equal to pH in the sea outside the cages (8.1-8.2). This corresponded to a median concentration of CO_2 of 2 and 4 mg/L at the two sites with CCS and ≤ 1 mg/L in net-pens and in the surrounding sea water. Inside CCS, measured pH ranged between 7.5 and 8.1 at site 1 and between 6.8 and 8.4 at site 3. The maximum concentration of CO_2 in CCS (pH = 6.8, cage no. 1, site 3) was >20 mg/L. This was recorded in October 2016 during a period with reduced water flow caused by accumulated sediments in the outlet.

Total ammonia Nitrogen (TAN) values from CCS were 0.3-0.5 mg/L. With salinity of 32.0 ppt, alkalinity between 2.2 and 2.3 mM and pH≥7.4 this corresponds with levels of toxic ammonia (NH_3) of less than 0.004 mg/L (Fivelstad et al., 1991). Levels of suspended solids (TSS) fluctuated between <8 and 169 mg/L. The highest concentrations of TSS inside the cages were recorded during March and April at site 1, a period with high TSS-values (100-200 mg/L) and high turbidity in the seawater around the cages.

Cross-sectional sampling within the cage volume in two CCS at site 1 (Supplementary data, Table 1, cages nos. 8 and 9), were performed at four depths (1, 3.5, 7 and 10 m) and at four different distances from the cage wall (1, 3.5, 7 and 11 m, where 11 m represented cage centre). Oxygen saturation ranged between 77 and 115% and was highest towards the surface, close to

the cage wall ($p \leq 0.001$ for both vertical and horizontal gradients). Temperature, salinity and pH showed no significant vertical or horizontal variation. However, the most prominent observation was the gradual reduction of pH from the cage wall towards cage centre. The total difference in pH between the cage wall (1 m) and cage centre (11 m) across all four samples in two CCS was 0.22 ($p<0.001$). This horizontal profile of pH and CO_2 is illustrated with data from April 2016 in Figure 10.

Figure 10

3.6 Histology of gills and kidneys

In the 2014 and 2016 year classes at site 1, gills, kidneys and pseudobranchia (less frequent) were sampled from the hatchery before sea transfer and at two to three sampling points during the seawater period. In the 2014 year class, 56% of the fish from the hatchery had mild to severe nephrocalcinosis. At sea transfer of the 2016 year-class, 27% of the smolt at the hatchery had mild kidney lesions compatible with nephrocalcinosis. Only a few individuals with mild signs of nephrocalcinosis were found during the seawater period, indicating no further development of kidney lesions and perhaps even an improvement at sea. All gills were normal from smolt sampled at sea transfer. After the seawater period from October 2014 to April 2015 and from October 2016 to April 2017, all gills from post-smolt in net-pens (2015) and CCS (2015 and 2017) had mild to moderate proliferative lesions. This coincided with a spring rise in plankton concentrations and increased turbidity in the sea water, a common feature of April at this latitude. Farm personnel also observed periods with reduced appetite, especially in the net-pens in April 2015. Lesions caused by the myxosporidian parasite *Parvicapsula pseudobranchicola* were present in a few of the pseudobranchia from fish in net-pens in 2015. In gills from CCS in April 2017 we also identified lesions involving costia (*Ichthyobodo necator*) and epitheliocystis-like inclusions (suspected *Branchiomonas cysticola*).

3.7 Sea lice

Sea lice counts from the first 4 CCS and the two net-pens were reported by Nilsen et al. (2017a). All sea lice counts in the other CCS showed zero salmon lice. *Caligus elongatus* were identified sporadically, and at a low prevalence (mean number of *C. elongatus* per fish between 0 and 0.1).

Discussion

The study covers a large and diverse data pool. All data on temperature, growth and mortality are reported as Supplementary data. The different topics (lice, water quality and water velocity, growth, mortality rates and mortality causes) are discussed below, with an emphasis on evaluating the possible connections between rearing conditions and biological results. We also evaluate the rearing environment and the biological outcomes in a fish welfare context.

4.1 Growth rates and FCR

The growth rate in terms of final weight and TGC clearly demonstrated improved growth in CCS compared to net-pens (2014-2015). A different outcome was reported in a study of production of Atlantic salmon one-year smolt (S1) in a raceway CCS (Balseiro et al., 2018), with higher TGC and condition factor (CF) in net-pens than in a raceway CCS. Based on the review from Thorarensen and Farrell (2011), a TGC between 2.7 and 3.0 should be anticipated in CCS, with values > 3.0 in more long-term studies. This is supported by growth data from an early CCS trial reported by Skaar and Bodvin (1993), where a trial of post-smolt Atlantic salmon between 60 and 700 g showed a TGC in CCS of approximately 3.5. The results from our study support the review of Thorarensen and Farrell (2011) and point towards the possibility of achieving higher growth rates with the optimisation of technology and farming methods. In comparison to net-pens, production of S0 in CCS also get an additional benefit from access to deep water with higher temperatures.

An important explanation variable for increased growth rate in CCS could be the water velocities and swimming speed. Nilsen et al. (2018) described a significant increase in growth and condition factor (CF) when water velocities were increased from 6 to 20 cm/s. This is the same range as the estimated water velocities in the cohort trial with CCS and net-pens in 2014-2015. Similar results, with more details on how muscle development responds to different swimming velocities are reported in recent studies of post-smolt salmon (Timmerhaus, pers.com). A moderate increase of water velocity and swimming activity leads to faster growth, more muscle development and increased CF, but no significant increase in visceral or muscular fat depostis (Jørgensen and Jobling, 1993; Solstorm et al., 2015; Nilsen et al., 2018). This is noteworthy because it is contrary to the common belief that farmed salmon with high CF are "fat and lazy". In this study, the final CF were lower in net-pens then the comparable CCS groups. We suggest that aerobic training and increased muscle growth was a major contributor

to the improved growth rates observed in CCS. Condition factor (CF) could be used to growth performance and welfare in Atlantic salmon during the seawater period. However, CF must always be interpreted in light of fish size, water velocities and the latest growth rate history.

Growth was recorded in each cage as weekly growth rates and as total growth rate for the entire production period. Notably, the estimates of end weight (W_1) are systematically less reliable than W_0, and this represents an important source of error when calculating growth rates in this kind of trial. As weighing procedures also represent a possible negative impact on fish welfare, the use of biomass frames or methods based on picture analysis of the swimming fish should be the standard procedure in future research and in supervision of commercial production.

We used TGC to compare growth rates of post-smolt across different production periods with variable temperature profiles. TGC is a growth model validated for use for fish between 100 and 3000 g and for water temperatures between 4 and 14 °C (Alanära et al., 2001); these conditions were met in our study and in the studies used for comparison. However, the latitude was not accounted for in the model, and a growth model incorporating the effect of day length, such as the Ewos Growth Index (EGI), could have been more appropriate (Aunsmo et al., 2014).

Seasonal variations could be another important bias in such growth studies. The specific growth rate declines with fish size and increases with water temperature within the temperature optimum of the species (Brett and Groves, 1979). A seasonal variation of TGC between 1.24 and 4.95 has been reported from studies of Atlantic salmon in net-pens (Mørkøre and Rørvik, 2001). Reduced growth of Atlantic salmon post-smolt at land-based farms during winter, despite stable water temperatures, indicates downregulation of growth during winter months with less daylight (Forsberg, 1995). The same tendency of depressed growth rates during winter has also been observed in Atlantic salmon >1000 g (Nordgarden et al., 2003). In the present study, data from net-pens was inconclusive. For S1 smolt in CCS the production period was from May to October without any winter season and with no observed seasonal trends. However, for CCS with S0, weekly TGC-values were reduced during January and February, before the growth rates stabilised during the spring months. There were too few cage observations and too many other confounding variables in this study to test the true impact of seasonal variations. However, seasonal variations and the impact of photoperiod could be important determinants for growth rate in CCS, and should be investigated further.

The feed conversion ratios (FCR) were calculated from total feed consumption and total increase in biomass. This does not account for possible loss of surplus feed; thus the calculated values are probably higher than if actual feed consumption of the fish could be used. This is common when using data of FCR from commercial farming to benchmark feed quality and feeding methods. FCR declines with increasing temperature, thus it also declines with increased SGR. At the same time, FCR increases with fish size (Brett and Groves, 1979). In this study, the moderate FCR values from 18 CCS with S0 correspond to the good growth rates with TGC around 3.0 and to the fact that the study was performed with salmon between 100 and 1000 g. The moderate FCR values also indicate efficient feeding systems with moderate loss of feed. However, the inaccuracies of the data material do not allow for detailed analysis of any group differences.

4.2 Mortality rates and mortality causes

We compared CM_{3mo} and the proportions of different mortality causes in our study with the data from Aunsmo et al. (2008) (Table 10). Cumulated mortality three months after sea transfer in our study was equal to or lower than in the net-pen study and the national reference data from S0 in 2006. Ulcers and fin rot represented around 50% of the total mortality after three months (91 days) in the net-pen study, again very similar to our data (although our scores were counted from CM_{total}, 159 days). Both studies showed a moderate prevalence of smolt quality problems, cachexia and physical trauma and a larger bulk of 'Other' causes of mortality. The prevalence of smolt quality disorders and physical trauma was zero in our S0 groups. However, the accuracy of the cause-specific mortality records performed by trained professionals in the study of Aunsmo et al. (2008) was probably higher than our study where we had to rely more on farm data.

Some of the mortality in S0 groups classified as 'Other' during the period in seawater could have been caused by gill lesions. Gill pathology developed during the seawater period both in 2014/2015 and 2016/2017. Lesions could be caused by epithelial irritation or damage from high plankton concentrations; more specific pathology caused by specific gill pathogens could also be a contributing factor.

Table 10

In the first study of CCS by Skaar and Bodvin (1995), total cumulated mortality (CM_{total}) was

lower in CCS (1.3%) than in the net-pen (3.6%) over a period of 5 months after sea transfer. This situation is partly explained by three bath treatments with organophosphates against salmon lice in the net-pen cage during the trial period. In a study of S1 smolt (Balseiro et al., 2018), CM_{total} was similar in the CCS raceway system (1.3%) and the net-pen (1.0%) after a trial period of 4 months. Although other commercial scale CCS studies are mostly pilots with few replicates and few details on mortality and mortality causes, they support the results from our trials, showing low mortalities during the production of post-smolt Atlantic salmon in CCS.

The Institute of Marine Research (IMR) and the Norwegian Veterinary Institute (NVI) have suggested different levels of fish welfare in terms of total mortality during seawater production of salmon (Svåsand et al., 2016; Grefsrud et al., 2018). The median (with 25^{th} and 75^{th} percentiles) cumulated mortality of Atlantic salmon the first three and five months after sea transfer in the period between 2009 and 2015 is compared to our CCS data in Table 11. The cumulated mortality in 23 CCS is compared to the 25^{th} and 75^{th} percentile levels in the national data in Table 12. When comparing with the data from Aunsmo et al. (2008) and IMR, cumulative mortality rates from CCS in our study were considered equal to or lower than cumulative mortality rates described from commercial net-pens.

Table 11

Table 12

Ulcers and fin rot

The main cause of mortality, as measured in terms of proportion of total mortality or as the diagnosis affecting most cages, was ‘Ulcers’, composed of skin ulcers and fin rot. Ulcers are considered a common disease in Norwegian salmon and rainbow trout farming, with a negative impact on fish welfare, economic loss because of mortality and reduced fish quality at harvest (Takle et al., 2015). Fin lesions are common on all cultivated fish delivered from hatcheries, with suggested principal factors including: overcrowding, malnutrition, poor water quality, abrasive rearing surfaces and bacterial infections (Bosakowski and Wagner, 1994; Latremouille, 2003; Ellis et al., 2008). In a recent study of post-smolt salmon after sea transfer, thickness of the skin and mucus layer will increase gradually, parallel to a temporary immune suppression, during the first month (Karlsen et al., 2018). Karlsen et al. (2018) also compared skin health and development of immunocompetence in salmon from net-pens and a raceway CCS, with the

conclusion that any differences between the two systems were connected to the different temperature profiles and not to the rearing systems. They also showed that during the first month after sea transfer Atlantic salmon post-smolt are particularly susceptible to skin lesions and/or infections. In our experience, the prevalence and severity of skin and fin lesions during the first period after sea transfer may also be aggravated if the smolt group is partially unsmoltified or if stress or mechanical trauma was inflicted during transport from the hatchery to the sea site. After sea transfer, there are several management procedures that could cause skin lesions and thus ulcer development, such as sea lice (if in high abundance), sea lice counts with associated crowding and anaesthesia (Mejdell and Nilsen, 2016) and sea lice treatments (Overton et al., 2018). In this study, there were no sea lice in CCS, and thus no sea lice treatments. From our observations and health investigations throughout the trials, there were few possibilities for mechanical trauma to the fish in the post-smolt period between stocking and emptying of the cages. Crowding of fish (especially in cages with high water velocities) and the use of dip-nets during sea lice counts and routine weight monitoring were regarded as the two management practices that were most likely to have a negative impact on skin and fin conditions of the fish. During winter, at low water temperatures, the frequency of such procedures was reduced, to spare the fish from physical trauma and secondary bacterial infections.

The manifestation of ulcers and fin lesions during rearing in sea-water cages was diverse. This reflects the variation of pathogens involved, and the complex interaction between fish, pathogens and the environment. A defined diagnosis in Norwegian salmon farming is 'winter ulcer', first described by Lunder et al. (1995), with the involvement of the pathogenic bacteria *Vibrio visocosa* (now: *Moritella viscosa*) and *Vibrio wodanis* (now: *Aliivibrio wodanis*). Of these two, *M. viscosa* has been considered the most important pathogen, and most salmon are today vaccinated with oil-based vaccines containing *M. viscosa*. More recently, bacteria of the *Tenacibaculum* species have been isolated from severe outbreaks of ulcer-related mortalities, especially in Northern Norway (Olsen et al., 2011; Småge et al., 2015; Olsen et al., 2017). Often, a mixture of all these bacteria are identified during outbreaks of so-called 'winter ulcer' (Colquhoun and Olsen, 2019). In our study, the majority of bacteriological examinations of ulcers and fin lesions showed a broad variety of pathogens and possible pathogens, dominated by *Aliivibrio wodanis*, other unidentified *Aliivibrio* species and *Moritella viscosa*. A few *Tenacibaculum* sp. were isolated from fish with ulcers in cages 1 to 4; otherwise *Tenacibaculum* sp. did not appear to be of importance for the skin and fin lesions observed, as also verified by

negative smears from skin and ulcers (data not shown). Isolation of *Aliivibrio* species and *Moritella viscosa* from ulcers/fin lesions, kidneys and other organs and positive PCR-tests of *Moritella viscosa* (PCR protocols for *A. wodanis* were not developed) from both ulcers and kidneys indicate systemic infections, at least in the most severe cases. Pathological lesions observed in necropsies and histological samples support this. In all sampled fish, several pathogenic bacteria of the *Aliivibrio* species together with *Moritella viscosa* were a source of systemic infections, fin rot and ulcer development. Ongoing studies of CCS (unpublished data) and the magnitude of *Tenacibaculum* sp. mortality in Northern Norway in recent years (Colquhoun and Olsen, 2019) indicates that this could be an important pathogen to monitor in closed containment systems.

The pattern of mortality related to ulcers and fin rot differed between sites and smolt seasons. For off-season smolt (S0) at sites 1 and 3, 'Ulcers' peaked between week 17 and 22 after sea transfer, while for S0 at site 2 (net-pens) 'Ulcers' were observed throughout the period, without any obvious peaks. For one-year smolt (S1) at site 3, mortality came in two peaks; the highest peak occurred immediately after sea transfer (week two), with a new and more moderate peak around weeks 15 to 17. It seems reasonable to argue that the ulcer-related mortality observed during the first two or three weeks after sea transfer could be related to the general quality of the smolt. In all cases, ulcer-related mortality was associated with more than one bacterial pathogen or possible pathogen. The microbiological balance in the water and the interaction between fish and pathogens in the rearing environment continue to be important issues for the health and welfare of farmed Atlantic salmon, including when CCS is used.

Smolt quality

The most important drawback with smolt quality for the S0 groups was the high prevalence of nephrocalcinosis at sea transfer, with kidney lesions in approximately 25-50% of the fish in the two seasons this was investigated in (2014 and 2016). The histopathological score ranged from mild to severe, indicating periods during freshwater production with carbon dioxide levels above the recommended maximum levels of 10 to 15 mg/L (Fivelstad et al., 2003; Thorarensen and Farrell, 2011). This is a situation reported to be a problem in many Norwegian hatcheries (Gu and Olsen, 2019). Exposure to such levels of carbon dioxide could also lead to other physiological adaptations, to reduced growth rates in the initial seawater period (Martens et al., 2006) and possibly also to increased mortality. However, after sea transfer to cages with levels of CO_2 ≤2 mg/L, the kidney lesions seemed to disappear during the seawater period without

any significant mortality in any of the cages in our study. Mortality in the five S1 cages was high, with failed smolt quality as the most important mortality cause. The unsmoltified fish may also have been at special risk to the same factors that caused ulcers and fin rot, as suggested by Aunsmo et al. (2008). Lesions and stress during sea transfer could induce mortality and reduced performance (Handeland et al., 1996; Iversen et al., 1998), but this was not recorded as a significant problem in any of the groups in this study.

4.3 Water velocity, swimming speed, water quality

Water velocity is an environmental parameter with a large impact on fish growth, metabolism, behaviour and welfare (Palstra and Planas, 2011), primarily because higher water velocities induce more swimming activity. Increased water velocity improves fish growth (Leon, 1986; Jobling et al., 1993; Jørgensen and Jobling, 1993; Young and Cech, 1993; Davison, 1997; Castro et al., 2011; Ytrestøyl et al., 2017, Nilsen at al., 2018). Increased weight and CF with increased water velocity has been observed in several studies and is seen as a sign of increased muscle development (Totland et al., 1987; Kiessling et al., 1994; Castro et al., 2011; Solstorm et al., 2015). Low water velocity is in itself a possible negative environmental factor, because of increased frequency of negative social interactions (Solstorm et al., 2015) and slower recovery after stressful events (Veiseth et al., 2006). However, if water velocities are too high, it will lead to increased oxygen need and anaerobic metabolism with increased levels of lactate (Davison, 1997; Palstra et al., 2010) and finally to exhaustion, reduced growth and impaired fish welfare (Solstorm et al., 2015; Solstorm et al., 2016). In a study of low (6-8 cm/s) to moderate (19-21 cm/s) water velocities in 40m^3 models replicating the CCS described in this paper, Nilsen et al. (2018) showed increased growth and CF of Atlantic salmon in the moderate velocity group.

The dominant swimming behaviour in CCS in our study was a circular, counter-current schooling with swimming speed slightly faster than the water velocity. Thus, the true swimming speed was probably higher than the measured water velocities, especially for the largest fish. From our observations, most fish in the cages formed a 'doughnut' distribution with low density in the periphery and in the centre, and with detours to the surface during feeding cycles and as part of the usual rolling and jumping behaviour and to refill the swim bladder. With water velocities between 14 and 22 cm/s and fish lengths between 20 and 43 cm, estimated swimming speed would be ≥0.7-1.1 BL/s for smolt of 100 g and ≥0.3-0.5 BL/s for the largest post-smolt of 1000 g in this study. The optimal swimming speed for growth and welfare of Atlantic salmon

post-smolt (10 °C) has been reported as 0.8 BL/s (Solstorm et al., 2015) and the critical swimming speed (U_{crit}) for post-smolt (80-289 g) reported as 80-90 cm/s (Remen et al., 2016; Hvas et al., 2017). The swimming performance of salmon was also shown to be reduced when temperatures were raised to 23 °C or dropped to 3 °C. Thus, estimated swimming speeds in CCS in our study were within the described range for acceptable welfare and growth performance for post-smolt Atlantic salmon. However, the effect of swimming speeds closer to 0.8 BL/s for the larger post-smolt (500 to 1000 g) should also be studied. The effects of temporal and spatial variations in water velocity on swimming activity and schooling behaviour would also be of interest for the evaluation of fish welfare in such systems.

Minimum levels of oxygen saturation (DO) for sustained performance in salmon are highly dependent on temperature (Remen et al., 2013). Within the temperature range 7-13°C, no compromised metabolic rate and appetite are indicated in post-smolt Atlantic salmon at DO levels above 60% of saturation (Remen et al., 2016). Another study demonstrated negative impact on growth and feed utilisation in post-smolt salmon at 85% DO saturation compared to 100 % DO saturation at 8-9 °C (Bergheim et al., 2006). In the present study, mean DO was between 80 and 90% with lowest measured DO concentrations in CCS>71%. These oxygen concentrations were considered to be within safe limits for the welfare and growth performance of Atlantic salmon post-smolt (Thorarensen and Farrell, 2011; Remen, 2012), but it is important to emphasise the need for accurate monitoring and regulation of oxygen, especially at high temperatures and high production intensities. We discovered periods with larger oxygen variations and an increasing oxygen saturation towards the cage centre during cross-sectional samples at site 3 in trials after this study was finished. These variations were difficult to detect with the oxygen sensors located in the periphery, close to the cage wall. Future studies of oxygen consumption in CCS with a focus on diurnal variations, the impact of temperature, fish size, feeding rates and possible stressful events (fluctuations in rearing environment, crowding etc.) would be of interest to optimise oxygenation use and oxygenation systems in CCS.

After oxygen depletion, the accumulation of CO_2 is considered the next limiting water quality parameter in such flow-through systems. Thus, for the most part, water quality was measured between 12:00 and 16:00, at the time of day with assumed maximum impact of feed consumption and feeding activity on carbon dioxide production. Concentrations below 10-15 mg CO_2/l are recommended in salmonid culture (Thorarensen and Farrell, 2013; Fivelstad et al., 1995; Fivelstad, 2013). Throughout the study period, concentrations of CO_2 were usually

≤10 mg/L, and on a few occasions >15 mg/L. The high prevalence of nephrocalcinosis in kidneys from fish at sea transfer indicated high levels of CO_2 in rearing water at the hatcheries. The rapid recovery of kidney tissue after sea transfer to both net-pens and CCS indicates a successful regeneration of tubuli after restoration of adequate water quality.

A former study using the same CCS-technology (cage volume: 2870 m^3) concluded that a minimum specific water consumption (SWC) of 0.07-0.20 L/kg/min, corresponding to a feed load (FL) of 35-45 g feed/m^3, would control metabolite concentrations, with highest demand of water flow during summer temperatures (Nilsen et al., 2017b). In the current study, SWC at stocking was between 1.1 and 3.9 L/kg/min. At maximum production intensity, mean SWC was 0.27 L/kg/min (ranging from 0.20 to 0.50 L/kg/min). Mean maximum feed load (FL) was 21 g/m^3 (ranging from 11 to 30 g/m^3). These production intensities should provide sufficient water exchange to keep the accumulation of both CO_2 and ammonia below the suggested maximum levels. In our study, unionised ammonia (NH_3) also remained far below threshold levels, reducing the welfare and performance of salmon (maximum 12-25 µg NH_3/l) (Fivelstad et al., 1995; Thorarensen and Farrell, 2011). Although it is argued that SWC as low as 0.1 L/kg/min could provide sufficient metabolite removal (Forsberg and Bergheim, 1996; Nilsen et al., 2017b), other studies indicate that SWC≥0.2 L/kg/min is necessary to safeguard fish welfare (Calabrese, 2017) and SWC<0.3 L/kg/min was associated with the increased transcription of genes involved in skin health and immunity (Sveen et al., 2016). Further investigations of the relationship between SWC, water quality and fish welfare in commercial scale CCS are recommended.

From the start of the trials, there was no information available about how the temperature of water pumped from a depth of 25 m would be influenced by temperatures in the air and the water surrounding the floating tarpaulin bag. During cross-sectional sampling in two CCS at site 1 in 2016, there were no spatial variations in temperature or salinity. Sampling in several CCS around the year and under different temperature conditions confirmed the homogeneity of temperature and salinity in the whole volume of CCS (unpublished data). We also started out with the assumption that if water velocities, oxygen saturation, CO_2/pH, TAN and suspended solids fluctuated throughout the cage volume, it would be with a significant vertical gradient. The data from samples of TAN and suspended solids are too limited and inconclusive to support any theories about cage profiles. However, the horizontal gradient of water velocity, CO_2 and pH is important for understanding water circulation and water quality. The spatial variation of

731 water velocities, pH and CO_2 concentrations described in this study are also consistent with
732 more detailed studies performed in the CCS at site 3 during 2017 and 2018 (unpublished data).
733 It is our recommendation that studies on the rearing environment in this kind of CCS should
734 always investigate the vertical and horizontal gradients of both water velocity and water quality.
735 The magnitude and importance of diurnal fluctuations (Bergheim and Fivelstad, 2014; Kvamme
736 et al., 2018) should also be more thoroughly investigated.
737
738 *4.1 Sea lice*
739 The first published study from trials with this CCS project (Nilsen et al., 2017a) showed that
740 CCS technology with water inlets ataround 25 m provided effective protection against salmon
741 lice. The total absence of *Lepeophteirus salmonis* from all CCS in the present study supports
742 this conclusion. This is also in accordance with studies modelling the vertical dispersion of
743 salmon lice larvae (Samsing et al., 2015, Johnsen et al., 2014; Johnsen et al., 2016) and studies
744 on the use of artificial light treatment (Hevrøy et al., 2003), sea lice skirts (Grøntvedt et al.,
745 2018; Stien et al., 2018) and so called 'snorkel' cages (Stien et al., 2016). All these studies
746 indicate that deeper water provides better protection against salmon lice, with a vertical
747 threshold where the density of larvae becomes so low that infestation of salmon becomes
748 unlikely. The vertical distribution of larvae of *Caligus elongatus* is less studied. Sporadic
749 occurrence of adult *C. elongatus* on the salmon in CCS in this study and Nilsen et al. (2017a)
750 could be caused by adult parasites living on marine fish (Heuch et al., 2003) around the cages,
751 but jumping off their hosts (Schram et al., 1998) and entering the water inlets.
752
753 *4.6 Fish welfare considerations*
754 The study combined animal-based welfare indicators such as mortality, mortality causes and
755 growth rates, with resource-based welfare indicators such as water velocity, temperature,
756 oxygen, carbon dioxide and other water quality parameters. These indicators are particularly
757 appropriate for an evaluation of fish welfare carried out from a function-based perspective
758 (Fraser, 2003), emphasising biological functioning measured as health condition, production
759 parameters and basic environmental factors. The affective state and the behavioural preferences
760 of the fish were not investigated in our study. The most positive results from a fish welfare
761 perspective were (1) no salmon lice and no lice treatments in CCS, (2) homogenous and stable
762 rearing conditions within the limits of acceptable fish welfare, (3) high growth rates, and (4)
763 low mortality rates in S0 groups and (5) a good reproducibility of these results over time and
764 with different sites and cage designs involved.

The critical lessons learned were: (1) high mortality in S1 groups was caused by smolt quality problems, leading to poor welfare not only for the fish that died, but probably also for a larger proportion of the fish during the initial period after sea transfer, (2) ulcers and fin rot caused by bacterial infections represent a risk of depressed feed intake, reduced growth rates and compromised fish welfare in a large proportion of fish groups even at moderate to low mortality rates, (3) high water velocities in CCS could represent a problem for welfare-friendly handling of fish during crowding and sampling; hence velocities must be reduced before crowding begins, (4) the maintenance of a stable and fish-friendly rearing environment requires a high level of technical and biological qualifications for all personnel involved in the daily management, (5) it is necessary to maintain sufficient water circulation and water quality during the systematic removal of biofouling from water inlets and the inside of bags, (6) more knowledge about the fish skin barrier is needed to develop better prophylactic measures to improve skin health, and finally (7) more effort should be directed towards understanding the interaction between fish, rearing conditions and the bacterial community with a focus not only on ulcers and fin lesions but also on growth and fish welfare.

4.7 Conclusions

Production of post-smolt Atlantic salmon in closed containment systems (CCS) showed high growth rates and low to moderate mortality rates. Mortalities caused by 'Ulcers and fin rot' (various bacterial infections) and 'Failed smolt' were the two most important specific mortality causes and fish welfare issues in CCS. It was possible to maintain water flow, oxygen saturation and water quality within safe limits for fish health and welfare. Comparison with post-smolt salmon in net-pens indicated that the increased water velocities in CCS could enhance muscle development and thus fish growth. With production of off-season smolt, access to warmer water during the coldest season (October to April) was seen as an added value to growth rates and fish welfare of post-smolt in CCS. With the use of deep water (25 m) in CCS, it was also possible to effectively prevent infestation with salmon lice (*L. salmonis*).

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Figure 1

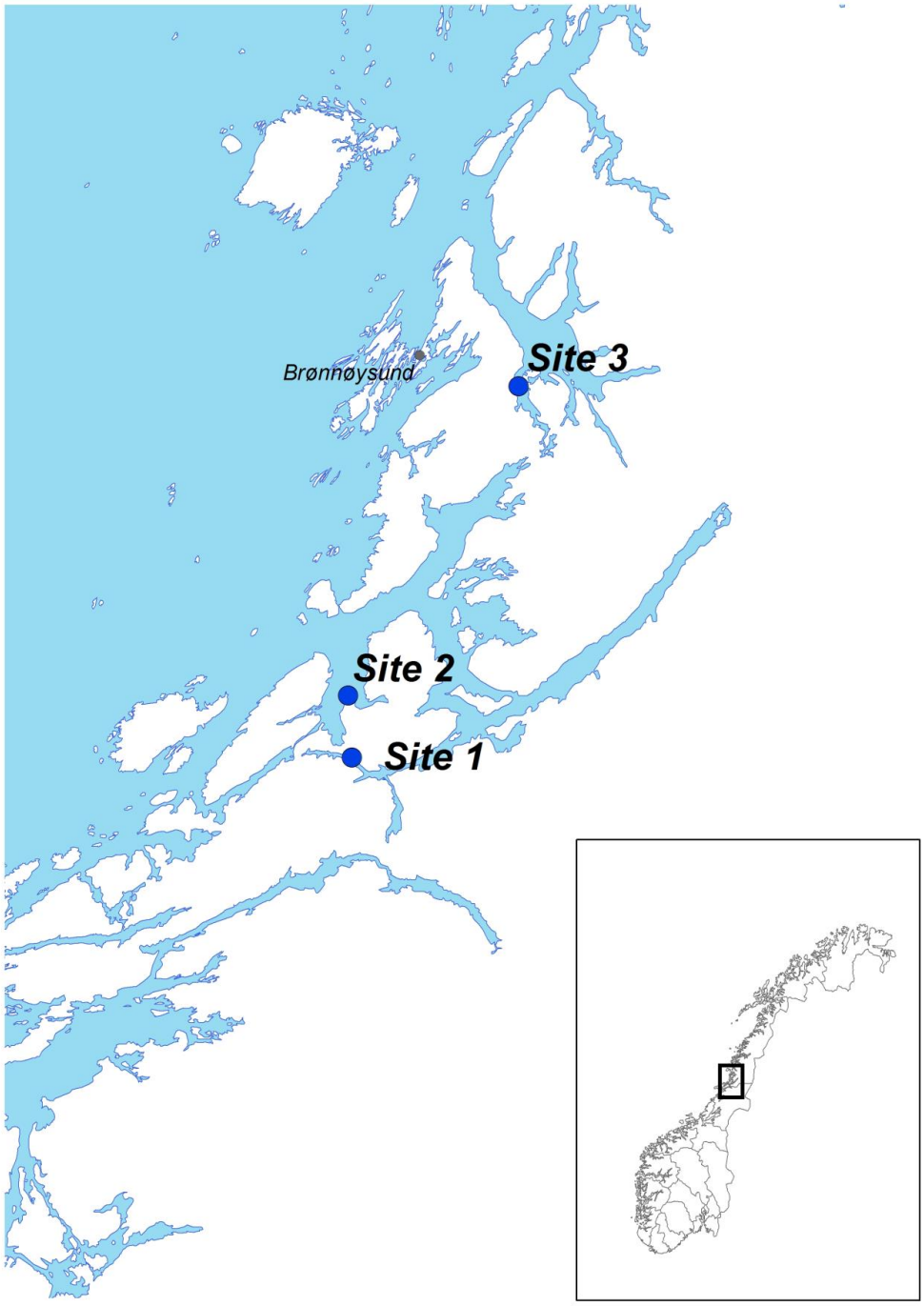
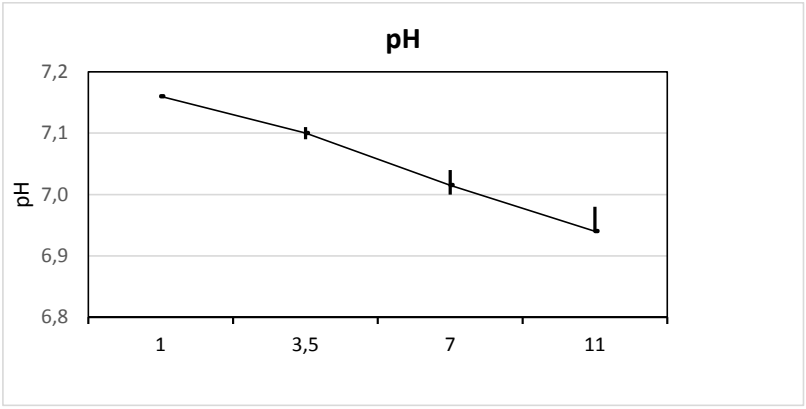


Fig. 10


pH
7,2
7,1
7,0
6,9
6,8
pH
1
3,5
7
11

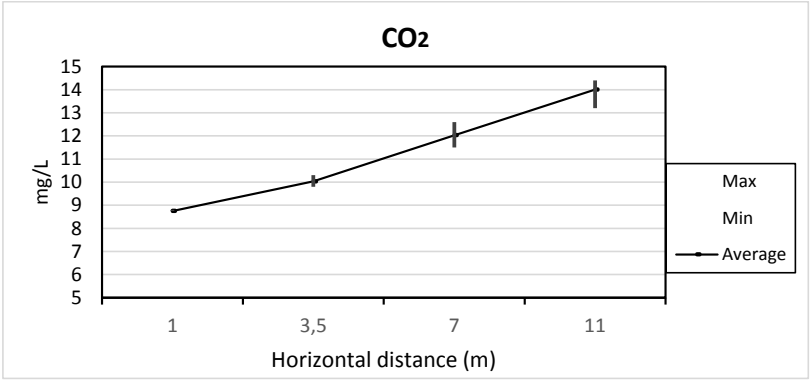

CO2
15
14
13
12
11
10
9
8
7
6
5
mg/L
1
3,5
7
11
Horizontal distance (m)
Max
Min
Average

Figure 2

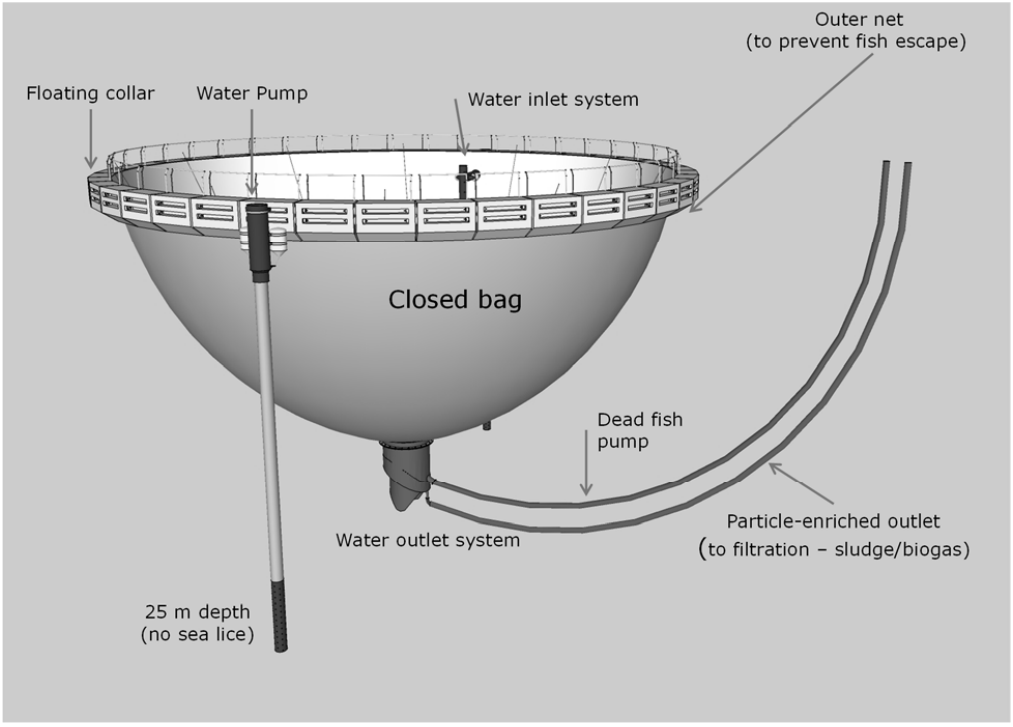

Outer net
(to prevent fish escape)
Floating collar
Water Pump
Water inlet system
Closed bag
Dead fish
pump
Particle-enriched outlet
(to filtration – sludge/biogas)
Water outlet system
25 m depth
(no sea lice)

Figure 3

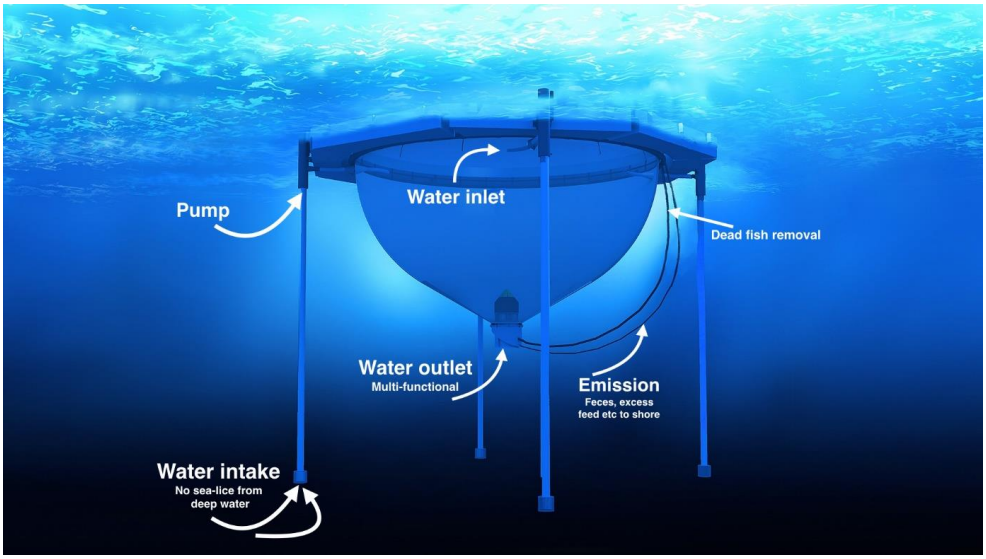
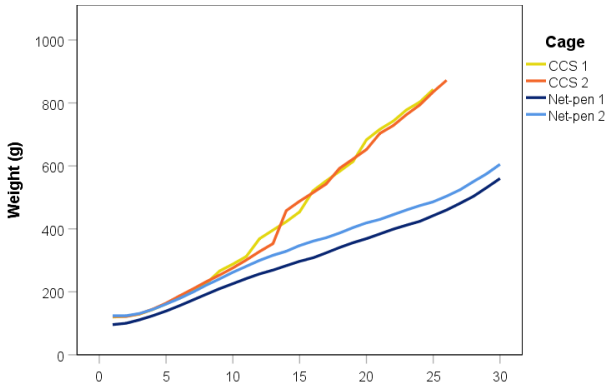
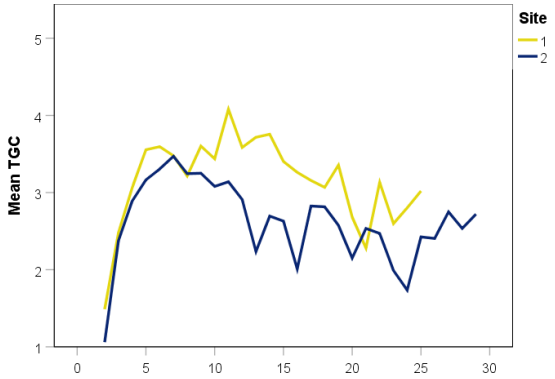

Pump
Water inlet
Dead fish removal
Water outlet
Multi-functional
Emission
Feces, excess
feed etc to shore
Water intake
No sea-lice from
deep water

Figure 4

Figure 5


Weight (g)
1000
800
600
400
200
0
0
5
10
15
20
25
30
Cage
CCS 1
CCS 2
Net-pen 1
Net-pen 2


Mean TGC
5
4
3
2
1
0
5
10
15
20
25
30
Site
1
2

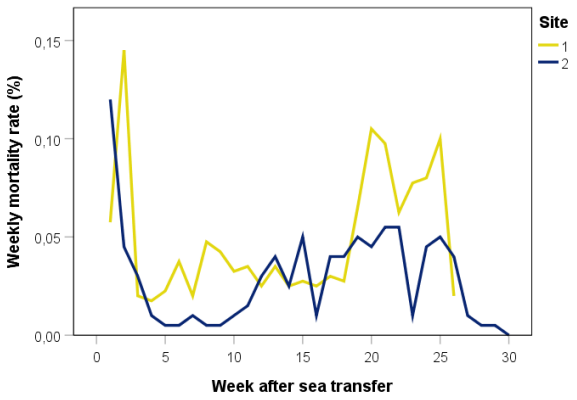

Weekly mortality rate (%)
0,15
0,10
0,05
0,00
0
5
10
15
20
25
30
Week after sea transfer
Site
1
2

Figure 6

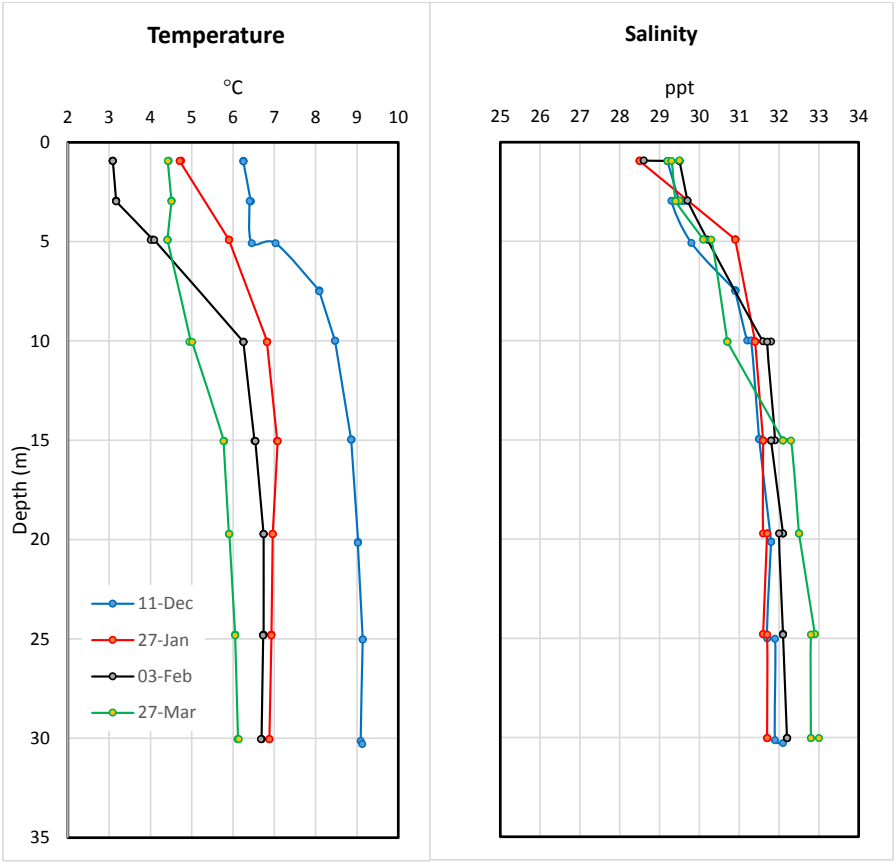

Temperature
°C
2 3 4 5 6 7 8 9 10
0
5
10
15
20
25
30
35
Depth (m)
11-Dec
27-Jan
03-Feb
27-Mar
Salinity
ppt
25 26 27 28 29 30 31 32 33 34

Figure 7

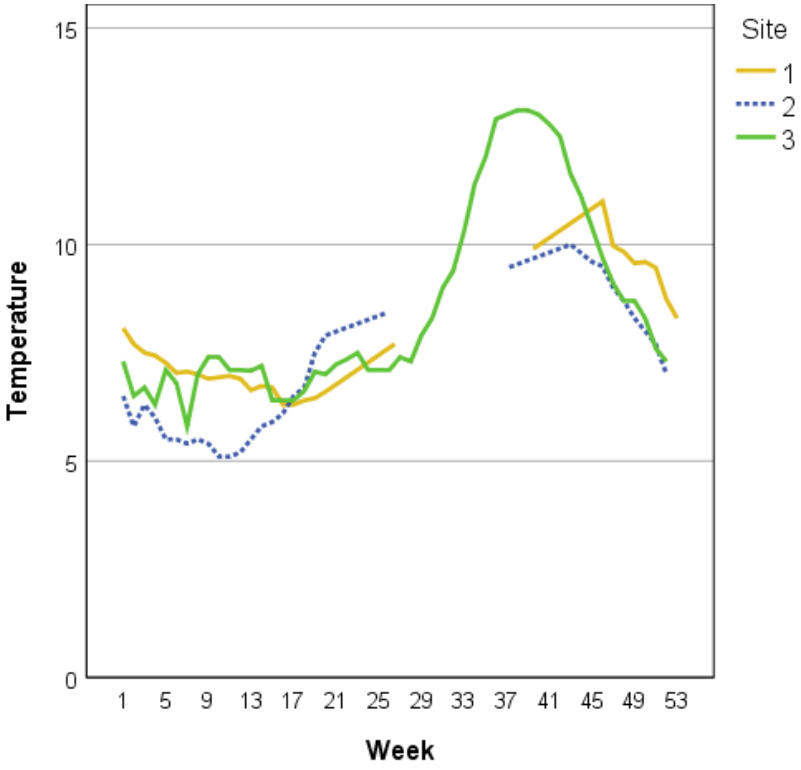

Site
1
2
3
Temperature
15
10
5
0
1
5
9
13
17
21
25
29
33
37
41
45
49
53
Week

Figure 8

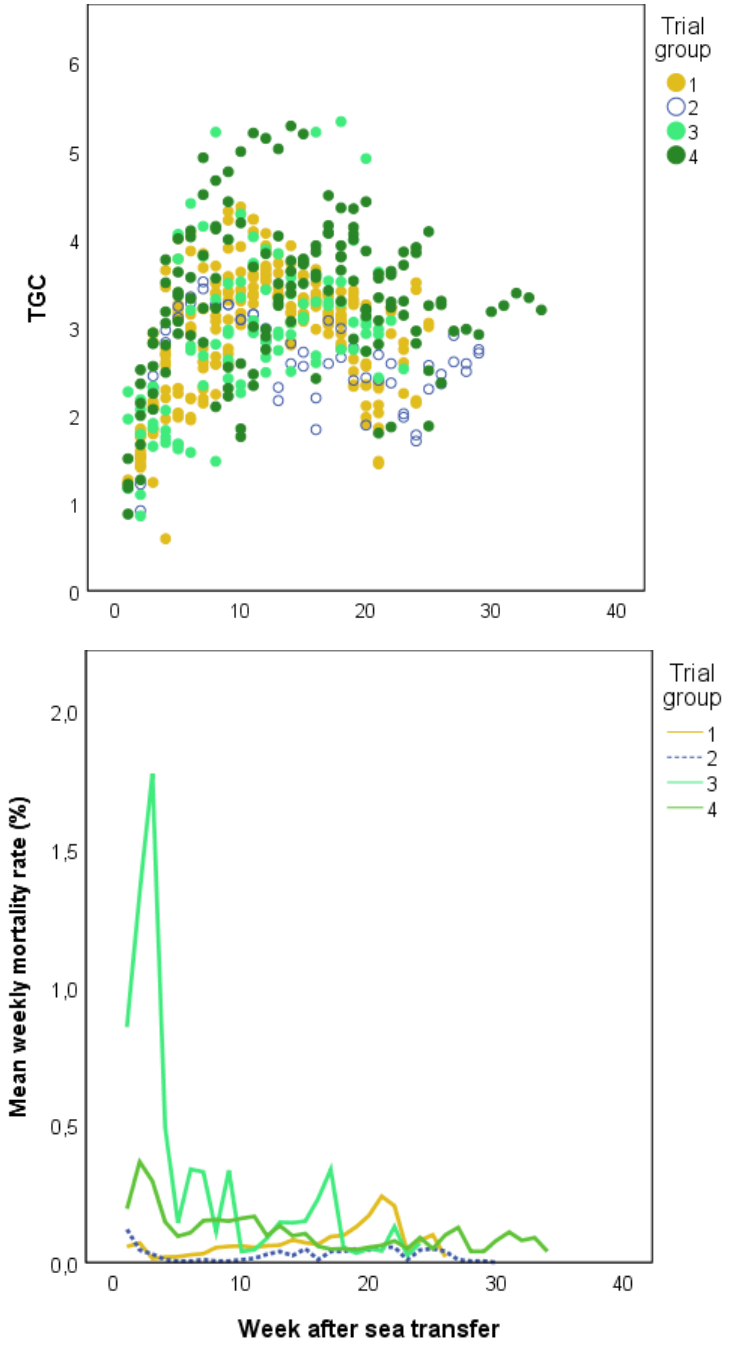
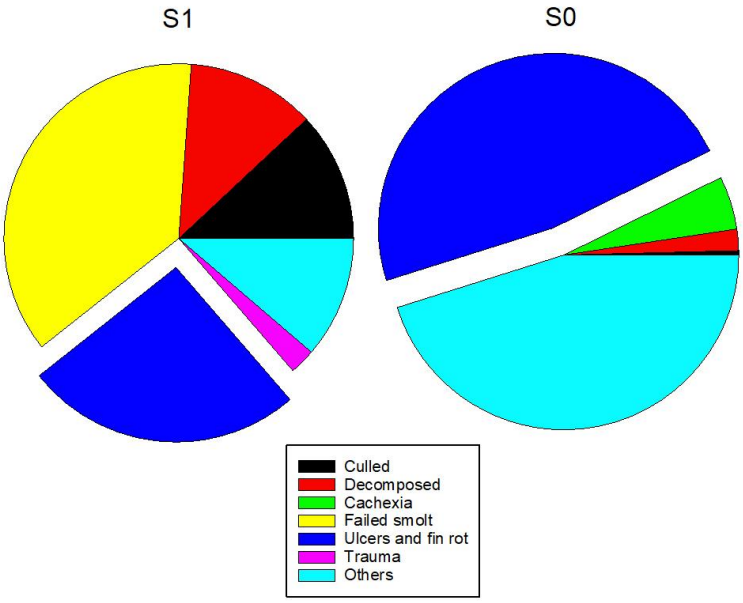

Trial group
1
2
3
4
TGC
0
1
2
3
4
5
6
0
10
20
30
40
Trial group
1
2
3
4
Mean weekly mortality rate (%)
0,0
0,5
1,0
1,5
2,0
0
10
20
30
40
Week after sea transfer

Figure 9


S1
S0
Culled
Decomposed
Cachexia
Failed smolt
Ulcers and fin rot
Trauma
Others

Figures Captions

Figure 1

Location of sea sites in Brønnøy and Bindal, Nordland county. Site 1: research site with CCS (2014-2017), site 2: commercial site with net-pens (2015-2016), site 3: research site with CCS (2016-2017) (Illustration: A. Tarpai).

Figure 2

Design of a closed, tarpaulin CCS, 2870 m^3 volume. Water inlet at 25 m depth through a 25 mm filter. Effluents separated into three fractions: water, sludge and dead fish. A net (not shown in the figure) surrounded the cage and the tubes to prevent escapees. (Illustration: AkvaFuture AS).

Figure 3

Design of the larger CCS used at site 3, volume 6000 m^3. The same basic design as the smaller cages, also here with a net (not shown) surrounding the cage and tubes (Illustration: AkvaFuture AS/Visual 360).

Figure 4

Site 3 with 10 CCS, each with a volume of 6000 m^3 (Photo: AkvaFuture AS).

Figure 5

Comparison of weight, mean thermal growth coefficient (TGC) and mean weekly mortality rates (%) between two CCS (site 1) and two net-pens (site 2), October 2014 to May 2015. All data are plotted against week after sea transfer. Upper panel: stocking weight (g) in all four cages, central panel: mean weekly TGC (sites 1 and 2), lower panel: mean weekly mortality rate (sites 1 and 2).

Figure 6

Temperature (°C) and salinity (ppt) profiles in sea outside CCS at site 1, December 2014 to March 2015.

Figure 7

Seasonal fluctuation of water temperatures, October 2014 to May 2017. Three year classes and 12 CCS at site 1, one year class and two net-pens at site 2, two year classes and 11 CCS at site 3. At sites 1 and 2, fish were stocked from autumn to spring, at site 3 from May 2016 to May 2017.

Figure 8

Upper panel: Weekly TGC (upper panel) and mean weekly mortality rate (lower panel) of four trial groups, plotted against week after sea transfer. Trial group 1=CCS with S0 at site 1 (n=12), group 2=net-pens with S0 at site 2 (n=2), group 3=CCS with S1 at site 3 (n=5) and group 4=CCS with S0 at site 3 (n=6).

Figure 9

Cause-specific mortality recorded in CCS, October 2014 to May 2017. Left panel: one-year smolt (S1, n=5), right panel: off-season smolt (S0, n=18).

Figure 10

Variations in pH (median, range) and carbon dioxide concentration (mean, range) at 3.5 m depth from edge (1 m) to centre (11 m) of a CCS with 2870 m^3 volume, site 1, 1st April 2016.

Table 1

	2014		2015		2016		SUM	
	fish	cages	fish	cages	fish	cages	fish	cages
Net-pen S0	331,400	2	-	-	-	-	331,400	2
CCS S0	285,797	4	477,000	4	1,315,195	10	2,077,992	18
CCS S1	-	-	-	-	744,845	5	744,845	5
SUM	617,197	6	477,000	4	2,060,040	15	3,154,237	25

Table 2

		Net-pens			CCS		
	Sample	n	Mean	SD	n	Mean	SD
Weight (g)	1	80	106	22	63	112	21
	2	59	645**	122	41	747**	182
Length (cm)	1	80	21.5	1.5	63	22.0	1.5
	2	59	39.0	2.5	41	39.0	3.0
CF	1	80	1.01**	0.04	63	1.07**	0.05
	2	59	1.11***	0.08	41	1.25***	0.09

Table 3

	CCS vs. net-pens			
Parameter	Diff.	Low 95 % CI	High 95 % CI	p-value
Weight (g)	102	43	162	0.001**
Length (cm)	0.3	-0.7	1.4	0.547
Condition factor (CF)	0.13	0.09	0.18	<0.001***

Table 4

		CM_{3mo}							
		Total mortality		Cages					
Cage type	Season	n_0	%	n	Mean	SE	Median	Min	Max
Net-pen	S0*	331,400	0.3	2	0.3	0.1	0.3	0.3	0.4
CCS	S0*	113,380	0.6	2	0.6	0.3	0.6	0.4	0.8
CCS	S0	2,077,992	1.3	18	1.1	0.2	0.8	0.3	2.9
CCS	S1	744,845	6.2	5	5.9	1.7	3.3	1.9	10.6
CCS	Total	2,822,837	2.6	23	2.2	0.6	1.1	0.3	10.6

		CM_{total}							
		Total mortality		Cages					
Cage type	Season	n_0	%	n	Mean	SE	Median	Min	Max
Net-pen	S0*	331,400	0.9	2	0.9	0	0.9	0.8	0.9
CCS	S0*	113,380	1.4	2	1.4	0.2	1.4	1.2	1.5
CCS	S0	2,077,992	2.4	18	2.1	0.3	2	0.7	4.3
CCS	S1	744,845	7.2	5	7	1.3	6.7	2.9	10.9
CCS	Total	2,822,837	3.6	23	3.2	0.6	2.1	0.7	10.9

Table 5

Type	Smolt	Cages	n_0	Days	CM_{total}	1 Culled	2 Dec.	3 Cach.	4 Smolt	5 Ulcers	6 Trauma	7 Others
Net-pen	S0*	2	331,400	204	0.9	1.6	0	1.6	0	5.2	0	91.6
CCS	S0*	2	113,380	168	1.4	0	0	3.8	0	27.8	0	68.4
CCS	S0	18	2,077,992	161	2.4	0.4	2.0	4.9	0	47.5	0	45.1
CCS	S1	5	744,845	151	7.2	12.0	11.9	0	36.8	25.7	2.4	11.3
CCS	S0+S1	23	2,822,837	159	4.9	6.4	7.2	2.4	19.3	36.1	1.3	27.4
All	S0+S1	25	3,154,237	162	3.6	6.3	7.0	2.3	18.7	35.3	1.2	29.1

Table 6

		Temp (°C)					DO %					Salinity					pH				CO_2		
Site	Cage	n	Mean	SD	Min	Max	n	Mean	SD	Min	Max	n	Mean	SD	Min	Max	n	Median	Min	Max	Median	Min	Max
1	CCS	143	6.9	1.1	5.9	9.0	88	81	5.0	71	88	143	32.0	0.4	31	33	143	7.8	7.5	8.1	2	<1	4
1	Sea	78	6.2	1.4	3.1	9.1	48	95	9.2	81	108	78	31.1	1.4	27	33	78	8.1	8.0	8.4	<1	<1	<1
2	Net-pen	58	6.6	1.5	4.9	9.8	48	105	7.9	85	113	58	32.3	0.4	32	33	58	8.2	8.1	8.3	<1	<1	<1
2	Sea*	48	6.1	1.0	4.8	7.5	32	107	7.4	92	115	48	32.4	0.5	32	33	48	8.2	8.1	8.3	<1	<1	<1
3	CCS	130	8.4	2.3	6.4	13.0	122	86	4.4	77	102	130	32.6	0.4	31	33	130	7.5	6.8	8.4	4	<1	>20
3	Sea	173	9.3	2.9	5.5	14.0	165	95	9.0	81	131	172	31.6	1.3	23	33	173	8.3	7.8	8.5	<1	<1	2

Table 7

	W_0(g)	W_1(g)	Days	°C	kg/m^3	SGR	TGC	FCR*
n	23	23	23	23	23	23	23	18
Mean	104	637	159	8.0	17.3	1.14	3.04	1.10
SD	23	174	21	1.0	2.5	0.12	0.37	0.07
Median	103	533	154	8.1	17.3	1.10	2.90	1.09
Minimum	60	451	134	6.9	10.0	0.89	2.59	0.98
Maximum	164	1094	227	10.1	22.4	1.43	3.94	1.20

*: FCR from five CCS with S1 were excluded.

Table 8

	Cage type*	Weeks	Mean	SD	Median	Min	Max
t (°C)	Net-pen	60	6.9	1.6	6.4	5.1	10.0
	CCS	545	8.0	1.8	7.4	5.8	13.1
TGC	Net-pen	56	2.62	0.54	2.63	0.91	3.51
	CCS	482	3.04	0.80	3.13	0.59	5.33
Mortality (%)	Net-pen	60	0.03	0.03	0.02	0.00	0.12
	CCS	545	0.14	0.34	0.05	0.00	5.33

Table 9

				W			CF				% CF		
Year	Cage	Site	Time	n	Mean	SD	Mean	SD	Min	Max	≤0.9	0.9-1.1	>1.1
2014	CCS	1	1	125	104	20	1.10	0.07	0.98	1.41	0.0	55.2	44.8
	CCS	1	2	80	656	175	1.23	0.10	0.70	1.45	1.3	3.7	95.0
	Net-pen	2	1	80	106	22	1.01	0.04	0.92	1.11	0.0	96.3	3.7
	Net-pen	2	2	59	645	122	1.11	0.08	0.93	1.31	0.0	44.1	55.9
2015	CCS	1	2	34	511	157	1.20	0.13	0.75	1.47	2.9	11.8	85.3
2016	CCS	1	1	348	116	22	1.14	0.07	0.87	1.37	0.3	25.6	74.1
	CCS	1	2	406	505	128	1.18	0.10	0.89	1.47	0.2	19.5	80.3
	CCS	3	2	149	1063	252	1.29	0.12	0.93	1.60	0.0	4.0	96.0

Table 10

		CCS S0+S1	CCS S0	Net-pen S0	National data 2006
Number of fish (millions)		2.8	2.1	2.7	71.1
Number of sites		2	2	10	114
Number of cages (mean no.fish/cage)		23 (122,700)	18 (115,400)	20 (139,700)	667 (103,100)
Species		A. salmon	A. salmon	A. salmon	A. salmon and R. trout
Sea transfer period		08.05 - 21.12	16.10 - 21.12	28.8 - 26.11	1.8 - 31.12
Mean (SD) weight at sea transfer (g)		104 (23)	103 (19)	81 (25.8)	109.7 (43.2)[a]
CM_{3mo} (%)		2.6	1.3	2.1	3.7
Cm_{total} (%) (159 days)		3.6	2.4	n	n
Mortality causes (% of total mort.)	Cachexia	2.4	4.9	3.7	n
	Failed smolt	19.3	0	7.4	n
	Ulcers and fin rot	36.1	47.5	50.9	n
	Trauma	1.3	0	7.3	n
	Others	40.9	47.6	30.7	n

[a]Mean weight one month after sea transfer

Table 11

Source	Months	Median	25th	75th
National data 2009-2015	3	1,2	0,3	4,0
	5	1,8	0,6	5,7
CCS 2014-2017	3	1,1	0,5	2,4
	5	2,1	1,2	4,2

Table 12

Months	≤25	25-75	≥75
3	9 %	78 %	13 %
5	0 %	83 %	17 %

Captions Tables

Table 1

Number of fish and cages with one-year smolt (S1) or off-season smolt (S0) in two net-pens and 23 CCS at three different sea sites from October 2014 to May 2017.

Table 2

Weight (g), length (cm) and condition factor (CF) from cohort trial with post-smolt Atlantic salmon in two net-pens and two CCS, October 2014 to May 2015, n=number of fish in each sample. Mean (SD) values at start (sample 1) and end (sample 2) of the trial. **: p-value≤0.01, ***: p-value≤0.001.

Table 3

Comparison of weight, length and condition factor between two net-pens and two CCS, October 2014 to May 2015. Data were analysed with a mixed linear (ML) regression model and the differences reported with 95% confidence intervals and p-values.

Table 4

Upper: Cumulative mortality three months after sea transfer (CM_{3mo}), lower: cumulative mortality after ended trial period (CM_{total}). For the first column (Total) the population is total number of fish (n between 331,400 and 2,822,837), for the other columns (Cages) the population is cages (n between 2 and 23). The two CCS denoted with * are the cohort group from October 2014 to May 2015. These data are also included in the total account of CCS S0 cages (n=18) and CCS Total (n=23) in the rows below.

Table 5

Cause-specific mortality data from two net-pens and 23 CCS, October 2014 to May 2017. n_0 = total number of fish, t = time period (day), CM_{total} = total cumulated mortality. Mortality classified as (1) Culled, (2) Decomposed, (3) Cachexia, (4) Failed smolt, (5) Ulcer and fin rot, (6) Trauma and (7) Unknown or other (less prevalent) causes. The cages denoted with * is the cohort group from October 2014-May 2015. These data are also included in the total account of CCS with S0 (n=18) and CCS Total (n=23) in the rows below.

Table 6

Water quality at sites 1 and 3 (CCS) and site 2 (net-pens) inside cages and in the water column outside the cages (sea), October 2014 to May 2017. Number of measurements (n), mean (SD) temperature (^oC), dissolved oxygen (DO %) and salinity (ppt). Median pH (minimum and maximum). Measured at 1-10 m depth in the closed cages, at 1-25 m depth in net-pens and in the water column outside the cages. *: At site 2, monitoring of seawater outside the cages only between January and May 2015.

Table 7

Summarised production data from 23 CCS, October 2014 to May 2017. Start weight (W_0), end weight (W_1), number of days, mean temperature in °C, maximum density in kg/m^3, specific growth rate (SGR), thermal growth coefficient (TGC) and feed conversion ratio (FCR).

Table 8

Weekly production data from two net-pens and 23 CCS, October 2014 to May 2017. Temperature (°C), thermal growth coefficient (TGC) and weekly mortality rate (%). Reported as n (number of weekly registrations), mean, standard deviation (SD), median, minimum and maximum values.

*: Net-pens were compared to two CCS in a cohort trial (2014-2015) and the data presented here are from all 23 CCS, thus a direct comparison between net-pen data and CCS data in this table is not relevant.

Table 9

Sampled weights (W) and condition factor (CF) of post-smolt (<1000 g) in CCS and net-pens, October 2014 to May 2017, n=number of sampled fish. To the right: the proportion (%) of fish with CF≤0.9, 0.9<CF≤1.1 and CF>1.6. Time=1: start of trial, time=2: end of trial.

Table 10

Comparison of weight at sea transfer (g), CM_{3mo} and mortality causes in the present trial and a study of 20 net-pens with S0 Atlantic salmon from the 2006 year class (Aunsmo et al., 2008).

Table 11

Comparison of cumulated mortality rates in a national survey of production of Atlantic salmon in net-pens from 2009 to 2015 (Svåsand et al., 2016) and 23 CCS with post-smolt Atlantic salmon from 2014 to 2017. Reported as median mortality, 25^{th} and 75^{th} percentiles.

Table 12

Distribution of cumulated mortality from 23 CCS with Atlantic salmon post-smolt, grouped in three mortality categories defined by 25^{th} and 75^{th} percentiles of mortality in a national survey of production of Atlantic salmon in net-pens from 2009 to 2015 (Svåsand et al., 2016). Reported as distribution of CCS (% of cages) with mortality in the three mortality groups at three and five months after sea transfer.

III

Short communication

The impact of production intensity on water quality in oxygen enriched, floating enclosures for post-smolt salmon culture

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Key words: Floating enclosures; Salmon; Oxygen injection; Water quality; Intensification; Production capacity

Abstract

The main aim of the study was to decide the effect of specific water consumption (L/kg/min) and feed load per water flow (g/m^3) on the water quality parameters pH, CO_2, total ammonia nitrogen (TAN) and suspended solids (SS) in two large semi-closed containment systems (SCCS). The reported production parameters (range) in the two S-CCS were specific water consumption (q): 0.04-0.47 L/kg/min and feed load per water flow: 9.0-64 g/m^3. The study period was split in two sub-periods; January to May (4.4 -7.5 °C), and June to September (7.5-13.2 °C) before a regression model was used to determine the relationship between production intensity (q, feed load) and water quality (pH, CO_2). With the acceptable level of CO_2 defined as ≤10mg/L, the model predicted a minimum specific water consumption (L/kg/min) between 0.07 (winter) and 0.2 (summer). The predicted maximum feed load per water flow (g/m^3) was between 35 (summer) and 45 g/m^3 (winter). These calculated limits for production intensity were close to the values earlier reported for smolt or post-smolt production in large, onshore tanks.

1. Introduction

Norwegian salmon production is based on transference of smolt (size range 50–150 g) from land-based hatcheries to open net cages in sea water. The smolt are stocked in the cages in spring as one-year-old smolt (S1) or in autumn as out-of-season smolt (S0). A production cycle from hatching to harvest size of 5–6 kg normally takes between 24 and 32 months. Production

of post-smolt in closed systems could take place in sea water supplied on-shore recirculating farms (RAS), flow-through tanks or in floating enclosures in sea (DFO, 2008; Rosten et al., 2011). Floating semi-closed containment systems (S-CCS) are flow-through systems with pumping of water from the deep to avoid contamination with parasites and pathogens from the surface water levels. Such S-CCS could be introduced as an alternative to the traditional production cycle. Either as an intermediate stage for production of post-smolt of 500–1,000 g before transfer to open cages or to produce salmon until harvest size. With a prolonged on-growing in closed systems, it could be possible to achieve increased growth rate, reduced production period and improved feed utilization (Thorarensen and Farrell, 2011). Other possible benefits could be improved biosecurity, (Rosten et al., 2011), reduced waste loading (Braaten, 2017; Taranger et al., 2015) and reduced infestation of salmon lice (Nilsen et al., 2017).

In intensively run closed systems with oxygen injection, build-up of fish metabolites, especially carbon dioxide (CO_2) and ammonia (TAN), will set a limit to the lowest acceptable water flow (Sanni and Forsberg, 1996). Smolt producing on-shore tanks are usually equipped with systems for stripping of CO_2 (e.g. Summerfelt et al., 2000) but enclosed pilot sea-cages are so far run without such systems. As a general guideline, upper concentrations between 10 to 15 mg CO_2/l (Thorarensen and Farrell, 2011; Fivelstad, 2013) and 12 - 25 µg NH_3/l (Fivelstad et al., 1995; Knoph, 1995) at long-term exposure are recommended to avoid harmful effects in salmon.

There are few scientific studies describing operation of semi-closed systems for production of post-smolt (Rosten et al., 2011; Summerfelt, 2016). The main aim of this study was to determine the effect of specific water consumption (L/kg/min) and feed load per water flow (g/m^3) on the water quality parameters pH, CO_2, total ammonia Nitrogen (TAN) and suspended solids (SS) in two large semi-closed containment systems (S-CCS).

2. Methods

The study was performed with two S-CCS, both with a 2,870 m^3 cage volume (⌀=22 m, depth 13.5 m), supplied by non-filtered sea water from 25 m depth (Fig.1) as described in Nilsen et al. (2017). Two submerged propeller pumps (Xylem Norway AS, 5,5 kW) lifted the inlet water that was introduced at two points in the cage. The oxygen saturation was controlled by injection from an oxygen diffuser grid at 10 m depth. Two closed cages with minor technical

differences were used during a total of five management periods (cycles of stocking and emptying the cages) from January to September 2014. Three open cages (⌀=22m) at the same research site, and one commercial cage (⌀=51m) with a 35,000 m^3 volume at a neighbouring sea site, were stocked with the same cohorts of fish and monitored as parallels to the production (mortality and growth rates) in closed cages.

The project was licensed to produce fish within accepted limits of water quality and fish welfare, so experimental designs exploring extreme values of water quality was not possible. The water flow in each closed cage unit was fixed with little possibility of adjustment according to fish growth and increased fish density. Water quality data were recorded in the period 09.01.14-25.09.2014 from closed cages 6,8,9,12 and 13 and from open cages 11 and 14 (Table 1). These cages were part of a larger project with evaluation of closed cage technology (Nilsen et al., 2017) and the cage numbers in Table 1 refer to the different production cycles during the project period. Fish were transferred between the two closed cages in the period March 3rd to April 22nd because of technical maintenance, otherwise the group size in each cage was not manipulated (apart from mortality). Thus the recorded change of the independent variables specific water consumption and feed load was primarily a function of time and fish growth. The first data were recorded January 9th in closed Cage no. 6. The parallel open Cage no. 7 was then already harvested. Closed Cage no. 6 was split January 17th to closed cages no. 8 and 9 and open Cage no. 10 (on Site no. 3). The fish in closed Cage no. 8 and open Cage no. 10 was produced until harvest size (June to July 2014), the fish in closed Cage no. 9 was transferred to open Cage no. 11 on April 22nd and produced until harvest size (August 2014). Closed Cage no. 12 was stocked with post smolt April 30th, split into closed Cage no. 13 and open Cage no. 14 August 1st and then the fish in these two cages were produced until harvest size in January 2015.

Sedimentable particles (faeces, surplus feed) and dead fish were collected and pumped in separate tubes from the outlet to the surface. To prevent escapees, the entire tarpaulin with outlet and pipelines for sludge and dead fish was covered by a standard fish net (not shown in Fig. 1).

Fig. 1

2.1 Fish and feeding

The fish were Atlantic salmon (*Salmo salar*) of the Norwegian Salmo Breed strain from the same commercial hatchery (Bindalssmolt AS). The average fish size in the cages where water quality was examined ranged from 760 to 4,180 g and fish densities ranged from 9 to 39.6 kg/m^3. All groups were fed commercial pelleted feed (Skretting AS) according to the farms' standard operating procedures. The fish were fed at the surface by automatic feeders with two feeding intervals; from 08:00 to 09:30 and 13:10–16:00 (January to May), or 07:1510:20 and 15:30-16:45 (June to September), and with feeding 2/3 of the total day ration in the first feeding. Fish health was monitored with daily recording of mortalities, examination of dead and moribund fish and routine autopsies.

Growth rate was calculated as specific growth rate (SGR):

$SGR = 100 \cdot (\ln w_2 - \ln w_1)/t$

and as thermal growth coefficient (TGC):

$TGC = 1{,}000 \cdot (w_2^{1/3} - w_1^{1/3})/(T \cdot t)$

where w_1=initial weight and w_2=final weight (g), t=time (days) and T=average temperature (°C).

The mortality was reported in terms of % of the original number of stocked fish.

2.2 Flow and water quality

During the period January to September 2014, specific water consumption (q), feed load per water flow, pH and CO_2 was recorded in two S-CCS stocked with Atlantic salmon, through several test periods with varying fish number and size. The specific water consumption (q) was calculated by dividing estimated water flow (m^3/min) with biomass (kg). The water flow was estimated by recording the pump frequency (Hz), measuring the lifting height (cm) in the inlet tubes and converting this to a water flow in m^3/min, using a conversion table established by the authors. Information about fish size, biomass, feeding and mortalities were supplied from the farming journals.

Water quality was monitored in the closed cages at four depths (1-3-5-10 m) with 1–4 weeks intervals. Temperature, salinity, dissolved oxygen (DO) and pH were recorded with a handheld smarTROLL Multiparameter instrument (MP probe). Simultaneous monitoring of CO_2 was

performed with OxyGuard CO_2 Portable (minimum 10 minutes reaction time, accuracy documented by Moran et al., 2010). Temperature and DO were also continuously monitored and logged in all cages at the farm at 3 m depth (IQ Sensor net). During the test period, 41 samples of in-cage water were analyzed (lab: PreBIO AS, Norway) for pH (method: Norwegian Standard-EN ISO 10523), total ammonia nitrogen (TAN, method: Norwegian Standard-EN ISO 11732), and suspended solids (S-DM, method: Norwegian Standard-EN 872). To evaluate the quality of the test results from closed cages, parallel samples (n = 11) were taken from two open cages at the same site (Cages no. 11 and 14). Five day cycles in closed cages (07:00–21:00) were recorded to assess possible diurnal fluctuations. To calculate the models in this communication we use our measurements at 3 m with smarTROLL MP and Oxyguard CO_2 Portable, with most samples taken between 12:00 and 14:00 (range: 11:30-16:30). The depth of 3 m was used because (1) this corresponded with the localization of the cage sensors, (2) 3 m was evaluated to be a representative swimming depth of the fish during daytime, based on the routine visual inspections of the cages and (3) the repeated measurements from several depths in the closed cages showed little vertical variation of temperature, pH and CO_2.

2.4 Statistical methods

The recorded predictor variables were feed load per water flow (g/m^3), specific water consumption (L/kg/min), temperature (°C) and cage number. The outcome variables were pH and CO_2 (mg/L). The variables were analyzed with the statistical package SPSS (IBM, SPSS Statistics 21). After a preliminary analysis of causality and checking of the assumptions of normality, linearity, collinearity and homoscedasticity, it was decided that the two predictor variables had a strong collinearity and should be entered into the model separately. Because of the strong effect of temperature on fish metabolism, the study was split into two sub-periods; January to May (4.4–7.5 °C) and June to September (7.5–13.2 °C). The distribution of CO_2 values showed low normality and were replaced with ln-transformed values. The effect of cage was determined to be of minor influence, and removed from the final analysis. Within the two sub-periods, temperature still had a significant contribution to the regression analysis when testing q as the predictor value. When testing the effect of feed load, the temperature within each period had minor effect and was excluded from the analysis. We computed the regression analysis to assess the ability of feed load per water flow and the combination of q and temperature to predict in-cage values of pH and CO_2 in both periods. The fit curves were calculated and plotted with the use of mean temperature in the respective sub-periods. The

relationship between measured pH and CO_2 was plotted with an exponential regression curve calculated from a linear regression model with pH as predictor variable and $lnCO_2$ as outcome variable.

3. Results

3.1 Flow, feeding rate, CO_2 and pH

We recorded feed load per water flow, specific water consumption and water quality parameters 27 times in period 1 and 32 times in period 2, but lacked precise data about feed at four of the time points for recording in period 2. The oxygen saturation remained above 85% throughout the production cycle. The total water flow was 5.6-13.4 m^3/min, with a detention time of the water between 314 and 513 minutes. Fish density in the S-CCS was 9-39.6 kg/m^3. The number of recordings in the two S-CCS, median values (for pH), mean values and SD (all other parameters), minimum and maximum values for specific water consumption, feed load, pH, CO_2 (mg/L), $lnCO_2$ and temperature (°C) are summarized in Table 2. In the first period (January to May, n=27) the mean values and standard deviation (SD) were: q=0.21 L/kg/min (0.10) and t=5.7 °C (1.0). In the second period (June – to September, n=32) the mean values and SD were: q=0.34 L/kg/min (0.09) and t=9.5 °C (1.6). Median values of pH were 7.8 (Period 1) and 7.6 (Period 2).

Table 1

Table 2

Table 3

Then 8 regression equations were calculated to describe the association between the two independent productivity variables q (L/kg/min) and feed load (g/m^3) and the two outcome water quality variables, pH and CO_2 (Table 3). The recorded values of specific water consumption, feed load, pH and CO_2 in the S-CCS are plotted together with the calculated fit lines in Figures 2 and 3.

Fig. 2 a

Fig. 2 b

Fig. 3 a

Fig. 3 b

Concentrations of CO_2 throughout the period varied between 1 and 24 mg/L and pH varied between 6.8 and 8.2. The relationship between measured values of pH and CO_2 is shown in Fig. 4.

Fig. 4

3.2 TAN, NH_3 and suspended solids

Water samples from 3 m depth in semi-closed cages (n=41) and open net cages (n=11) were analyzed in a commercial laboratory. In S-CCS the pH was between 6.8 and 8.2, in open cages all values were ≥8.0. The concentration of TAN-N (mg N/l) in S-CCS was between 0.30 and 1.06, in open cages between 0.23 and 0.89. Calculated concentrations of un-ionized ammonia, NH_3, were < 10 µg N/L (equilibrium calculation based on Fivelstad, 1993). Levels of suspended solids (mg/L) in S-CCS were between <3 and 117, in open cages between <3 and 13.

3.3 Growth, mortality and fish health

The median value (minimum, maximum) for SGR in the five production periods in two SCCS was 0.62 (0.33, 0.69) and in the corresponding four open cages 0.65 (0.32, 0.83), TGC in S-CCS was 2.9 (2.4, 3.3) and in open cages 3.0 (2.2, 3.6), mortality in S-CCS was 3.1% (0.3, 24.4) and in open cages 7.4% (3.9, 28.9) (Table 1).

4. Discussion

Long-term exposure to elevated levels of dissolved CO_2 has been shown to cause reduced growth, increased FCR and nephrocalcinosis (Thorarensen and Farrell, 2011; Fivelstad, 2013). The knowledge about the effect of fluctuating CO_2 levels is scarce. In this study of a S-CCS pilot technology it was decided to use a restrictive limit of dissolved CO_2 ≤10 mg/L (Thorarensen and Farrell, 2011) to calculate the threshold values for minimum specific water consumption (L/kg/min) and maximum feed load per water flow (g/m^3).

The suggested minimum q required to maintain acceptable levels of CO_2 in the S-CCS (2,870 m^3) was ≥0,07 L/kg/min at low temperatures (4.4-7.5 °C), and ≥0.2 L/kg/min at higher temperatures (7.5-13.2 °C). The mean value for q (SD) in period 1 was 0.21 L/kg/min (0.10) and 0.34 L/kg/min (0.09) in period 2. This is in the same range as the specific water consumption, 0.12-0.49 L/kg/min, reported from commercial land based flow-through tanks (512-1,311 m^3) (Summerfelt et al., 2016). The suggested maximum level of feed load in this S-CCS-study was between 30 g/m^3 (period 2) and 45 g/m^3 (period 1), the mean and SD of the feed load in period 1 was 21 g/m^3 (12) and 20 g/m^3 (6) in period 2. These results are also within the same range as in the land-based flow-through tanks, with a reported feed load of 25-53 g/m^3 (Summerfelt et al., 2016). Fish density (kg/m^3), total flow per tank/cage (m^3/min) and maximum sustainable feed load (kg/d/tank) in this S-CCS-study were also within the same range as in the land-based flow-through tanks. The largest difference was the mean tank retention time. With a volume of 2,780 m^3, the S-CCS had a retention time between 314 and 513 minutes, while the land based tanks had a retention time between 35 and 171 minutes (Summerfelt et al., 2016). The figures from one 21,000 m^3 S-CCS are difficult to compare with our study, with an estimated total flow of 400 m^3/min, a flow per biomass of 1.0 L/kg/min, and a feed load of only 6.0 g/m^3 (Summerfelt et al., 2016).

The calculated levels of NH_3 in this study were stable with < 10 µg NH_3-N/L (equilibrium calculation based on Fivelstad, 1993), and without any risk of harmful effects on the fish. Without aeration of the water, any increase in production intensity leading to elevated levels of TAN would also increase the CO_2 levels and thereby cause a lower pH. In general, there is no risk of harmful concentrations of un-ionized ammonia (NH_3) in semi-closed cages at CO_2 concentrations above 10 mg/L due to reduced pH (< 7.2) and low dissociation of NH_4^+.

The fluctuating concentrations of suspended solids in S-CCS had a wider range, <3–117 mg/L, than the levels recorded in the open reference cages. There are few available reports describing effects of suspended solids (S-DM) on salmonids. Davidson et al. (2009) indicated 80 mg/L of suspended solids originated from fish and feed (feces, residual feed, biofilm) as a long-term upper limit. The levels of suspended solids are probably also influenced by tidal blooms of microalgae, and periods with high counts of phytoplankton were found in both open and closed cages. The levels of suspended solids in this study were not considered as high enough to cause any significant negative impact on the fish welfare, but also here it will be necessary to do more detailed studies.

Fluctuating temperatures, fish size and activity, feed supply, etc. resulted in varying pH and CO_2 concentrations at the same flow and feeding rate. This was also a pilot study, with limited possibilities to provide exact data about true water flow and how much of the supplied feed that was consumed and metabolized. These factors together with limited number of samplings, caused regression trends with moderate fitness (R^2: 0.40-0.90). Some rate intervals were weakly represented, especially at low flow/high fish stock during the winter and spring period (Fig. 3, Fig. 4). To collect information about these limits by systematically increasing production intensity in such large units beyond the limitations for acceptable fish welfare was regarded as unethical.

Usage of diffuser based oxygen injection to the cages made accurate estimates of oxygen consumption impossible. On average, the respiration quotient (RQ: mole CO_2 produced/mole O_2 consumed) in salmon is found to be approximately 0.9 (Bergheim and Fivelstad, 2014; Thorarensen and Farrell, 2011). Stoichiometrically, this corresponds to 1.0–1.2 mg CO_2 produced per mg O_2 consumed. Assuming an upper acceptable CO_2 concentration of 10 mg/L in the cages, the injected O_2 should not exceed around 8 mg/L without any CO_2 removal attempts. To optimize oxygenation and reduce the cost of oxygenation as much as possible, more specific studies on oxygen consumption in S-CCS is necessary.

Current velocity has a large impact on water quality and fish behaviour. When increasing density, the direct effect of accumulating metabolites can be mitigated by increasing the water flow rate, but only to a certain threshold. Increased water flow rate could lead to current velocities above the physiological limitations of the fish. For post-smolt this limit has been shown to be around 1.5 body lengths per second (BL/s) (Solstorm et al., 2015). The estimated current velocities in the semi-closed cages in this study were between 16 and 20 cm/sek, corresponding to 0.35–0.5 BL/s.

Based on a review study, Thorarensen and Farrell (2011) concluded that there appears to be no influence on growth, survival and welfare of Atlantic salmon at densities up to 80 kg/m^3. In recent tank studies it was shown negative effects on SGR, skin and on stress parameters with densities > 50 kg/m^3 (Sveen et al., 2016; Calabrese et al., 2017). In this study the densities were between 9 and 42 kg/m^3, and should not be the cause of any negative effect on fish growth or welfare.

Fish size, temperature and current speed explained 70% of the total oxygen consumption rate in post-smolt salmon stocked in commercial land-based flow-through tanks (Forsberg,

1994). The consumption rate in starving salmon was reduced by 30–40% (Grøttum and Sigholt, 1998), compared to normal oxygen consumption in active, fed salmon in aquaculture. At commonly applied continuous feeding in intensive salmon culture, e.g. distributed every 5 min for several hours per day, the max O_2 consumption rate is assumed to be 15–20% above the diurnal consumption mean (Thorarensen and Farrell, 2011). Such factors and others, e.g. stress impact at sampling, result in fluctuated CO_2 concentrations and pH within the same range of load level (flow and feeding rate). In this study, we found no indications of significant diurnal fluctuations of the values of pH, CO_2, suspended solids or TAN. This could be because samples were collected with too long time intervals, and more detailed investigations of this is necessary.

The connection between CO_2 concentration and pH in brackish water and seawater is dependent on the alkalinity/salinity of the water (Blancheton et al. 2007). A salinity of 32–34 ppt, characterizing the inlet water in the studied cages corresponds to an alkalinity of 2.0–2.2 meq/l. In this test, all samples had salinity between 31 and 33 ppt. When monitoring CO_2 in such systems, we recommend simultaneous measurement of temperature, salinity, pH and CO_2. Baseline studies of alkalinity and the total carbonate concentrations could also be useful if pH alone is used in the day-to-day monitoring in closed cages.

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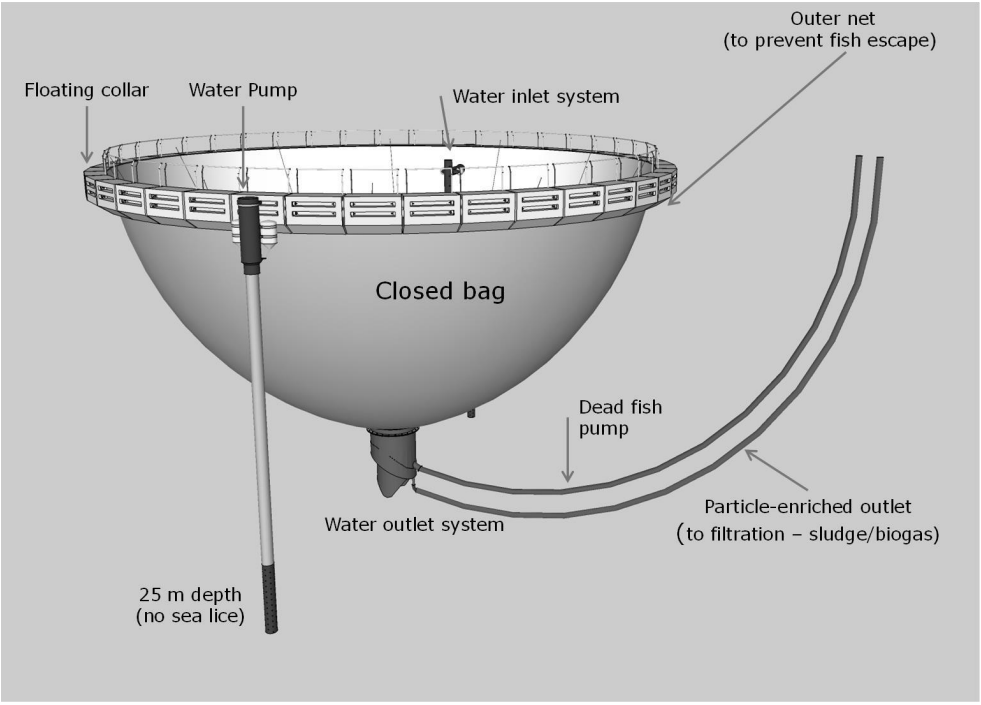

Outer net
(to prevent fish escape)
Floating collar
Water Pump
Water inlet system
Closed bag
Dead fish
pump
Water outlet system
Particle-enriched outlet
(to filtration – sludge/biogas)
25 m depth
(no sea lice)

Fig. 1.

Figure 2a

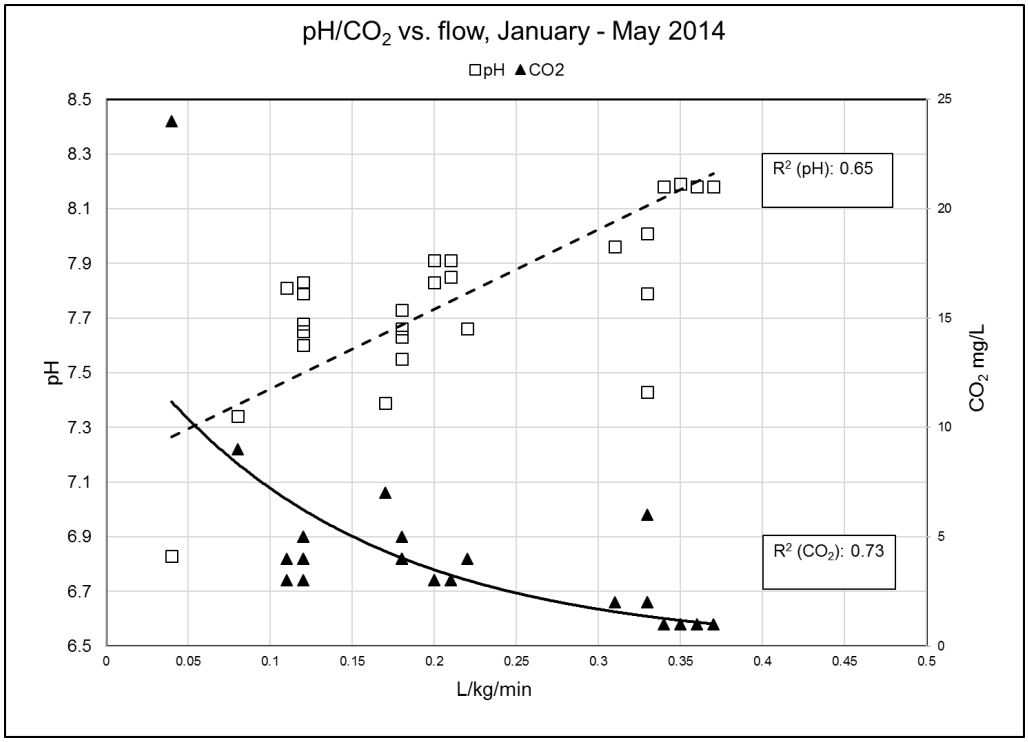

pH/CO2 vs. flow, January - May 2014
□pH ▲CO2
R2 (pH): 0.65
R2 (CO2): 0.73
pH
CO2 mg/L
L/kg/min
8.5
8.3
8.1
7.9
7.7
7.5
7.3
7.1
6.9
6.7
6.5
25
20
15
10
5
0
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
0.45
0.5

Figure 2b

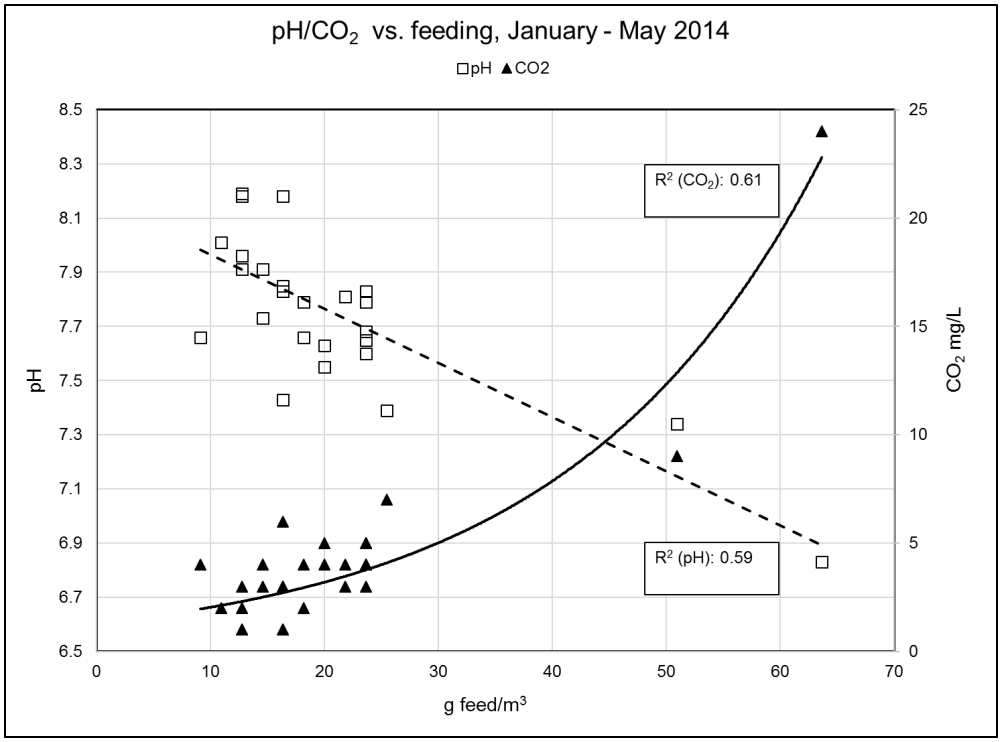

pH/CO2 vs. feeding, January - May 2014
□pH ▲CO2
R² (CO2): 0.61
R² (pH): 0.59
pH
CO2 mg/L
g feed/m3
8.5
8.3
8.1
7.9
7.7
7.5
7.3
7.1
6.9
6.7
6.5
25
20
15
10
5
0
0
10
20
30
40
50
60
70

Figure 3a

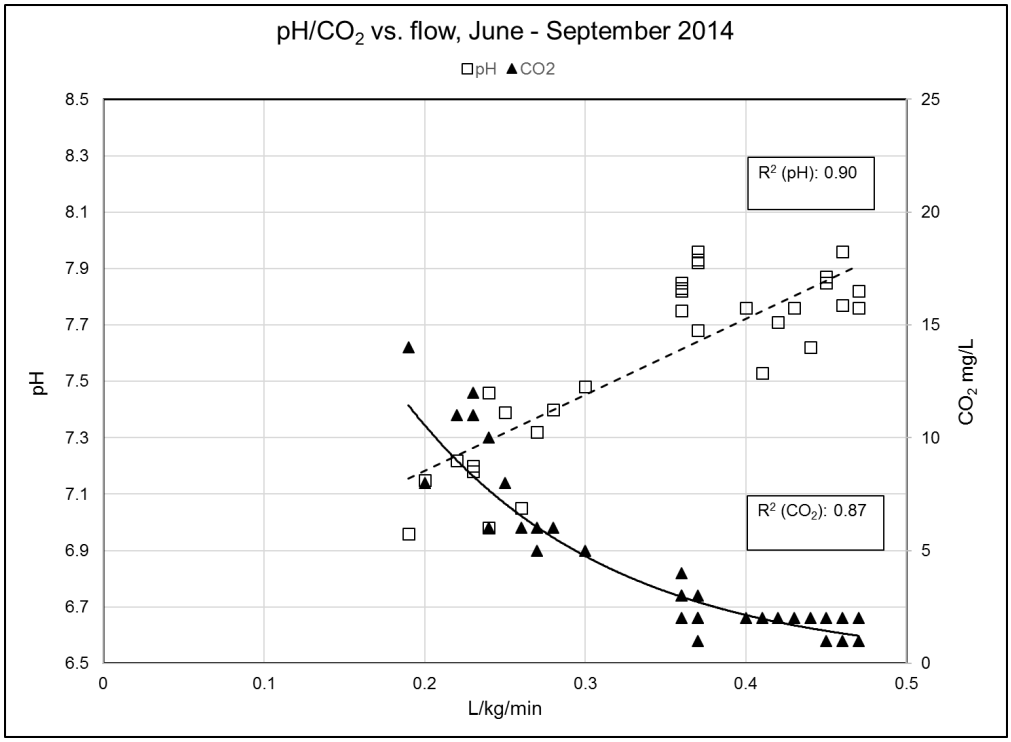

pH/CO2 vs. flow, June - September 2014
pH
CO2
R2 (pH): 0.90
R2 (CO2): 0.87
pH
CO2 mg/L
L/kg/min
8.5
8.3
8.1
7.9
7.7
7.5
7.3
7.1
6.9
6.7
6.5
25
20
15
10
5
0
0
0.1
0.2
0.3
0.4
0.5

Figure 3b

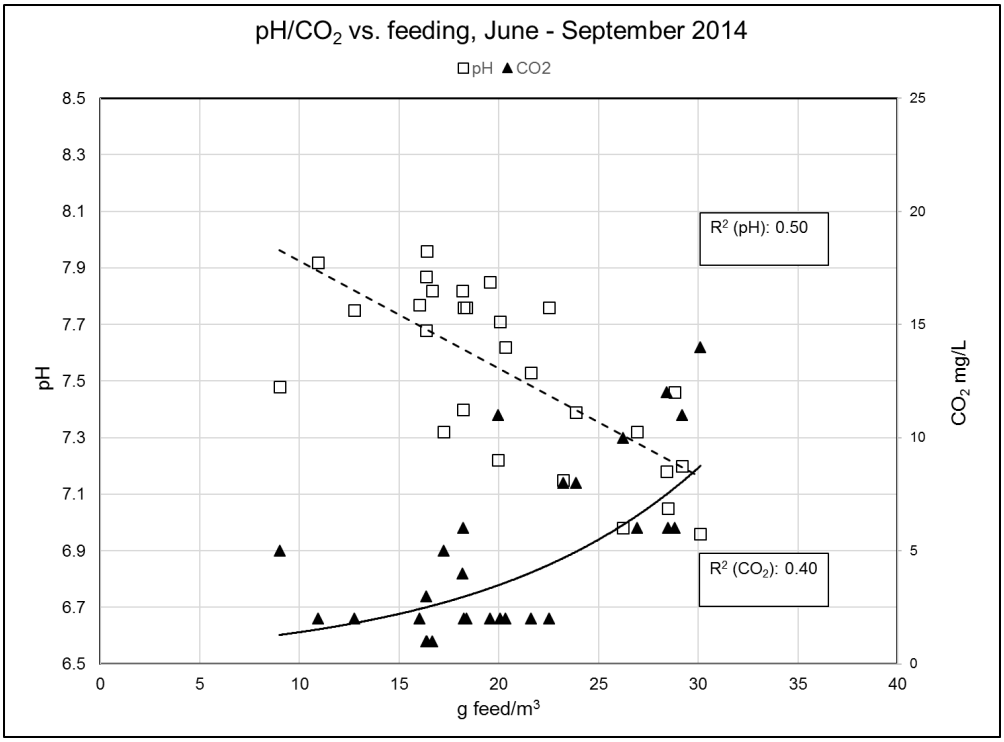
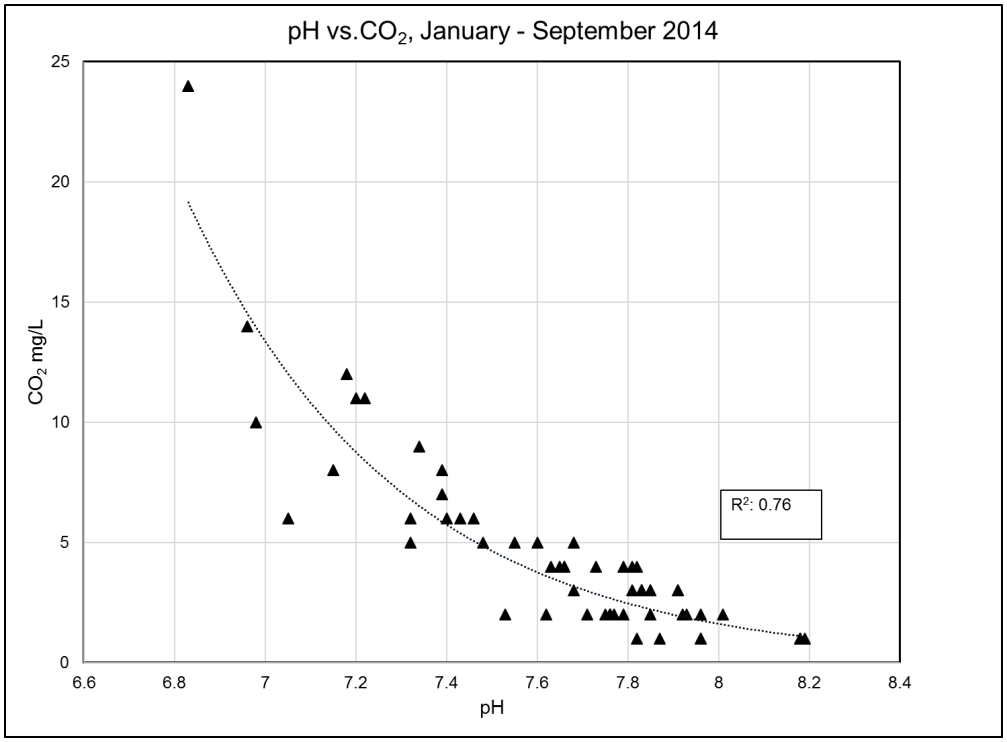

pH/CO2 vs. feeding, June - September 2014
pH
CO2
R2 (pH): 0.50
R2 (CO2): 0.40
pH
CO2 mg/L
g feed/m3
8.5
8.3
8.1
7.9
7.7
7.5
7.3
7.1
6.9
6.7
6.5
25
20
15
10
5
0
0
5
10
15
20
25
30
35
40

Figure 4


pH vs.CO2, January - September 2014
CO2 mg/L
pH
R2: 0.76
25
20
15
10
5
0
6.6
6.8
7
7.2
7.4
7.6
7.8
8
8.2
8.4

Captions Fig. 1. – Fig. 4.

Fig. 1.

Design of a closed, floating tarpaulin covered cage. Water inlet at 25 m depth through a 25 mm filter. Effluents separated in three fractions: water, sludge and dead fish. A net (not shown in the figure) surrounded the cage and the tubes to prevent escapees. (Illustration: Akva Design AS)

Figs. 2 a and b.

Concentration of carbon dioxide and pH versus (a) specific water consumption (q = L/kg fish/min) and (b) feed load per water flow (g feed/m^3) in sea water enclosures stocked with post-smolt salmon. January to May 2014 (4.4 – 7.5 °C). Solid lines are plots of the regression between CO_2 and (a) q (R^2 = 0.73) and (b) feed load (R^2 = 0.61). Dotted lines are plots of the regression between pH and (a) q (R^2 = 0.65) and (b) feed load (R^2 = 0.59).

Figs. 3 a and b.

Concentration of carbon dioxide and pH versus (a) specific water consumption (q = L/kg fish/min) and (b) feed load (g feed/m^3) in sea water enclosures stocked with post-smolt salmon. June to September 2014 (7.5 – 13.2 °C). Solid lines are plots of the regression between CO_2 and (a) q (R^2 = 0.87) and (b) feed load (R^2 = 0.40). Dotted lines are plots of the regression between pH and (a) q (R^2 = 0.90) and (b) feed load (R^2 = 0.50).

Fig. 4.

Plot of the relationship between measured levels of pH and CO_2. The dotted line is the calculated exponential regression line: CO2 = eksp(17,384- 2,113*pH), (R^2=0.76).

Table 1

Cage No.	Site No.	Cage type	N_0	N_1	W_0	W_1	Start Date	Closing Date	t	T	SR%	SGR	TGC
6	1	Closed	80,000	60,500	110	1,880	04.11.12	17.01.14	439	7.1	75.6	0.65	2.4
7	1	Open	20,000	14,211	110	4,030	04.11.12	10.01.14	432	7.7	71.1	0.83	3.3
8	1	Closed	10,700	10,092	1,880	3,810	17.01.14	10.07.14	174	6.1	94.3	0.41	3.1
9	1	Closed	27,300	27,224	1,880	2,570	17.01.14	22.04.14	95	4.9	99.7	0.33	2.9
10	3	Open	22,775	21,144	1,880	3,130	17.01.14	25.06.14	159	6.6	92.8	0.32	2.2
11	1	Open	27,224	25,192	2,570	4,330	22.04.14	01.08.14	101	9.4	92.5	0.52	2.7
12	1	Closed	33,194	32,915	740	1,320	30.04.14	01.08.14	93	7.9	99.2	0.62	2.6
13	1	Closed	18,545	17,968	1,320	4,180	01.08.14	16.01.15	168	9.2	96.9	0.69	3.3
14	1	Open	17,832	17,128	1,320	4,820	01.08.14	16.01.15	168	9.7	96.1	0.77	3.6

Table 2

Period	Parameter	n	Median	Mean	SD	Min	Max
1 (January – May)	q	27		0.21	0.10	0.04	0.37
	Feed	27		21	12	9	64
	pH	27	7.8			6.8	8.2
	CO_2	27		4	4	1	24
	$lnCO_2$	27		1.20	0.71	0.00	3.18
	t	27		5.7	1.0	4.4	7.5
2 (June – September)	q	32		0.34	0.09	0.19	0.47
	Feed	28		21	6	9	30
	pH	32	7.6			7.0	8.0
	CO_2	32		5	4	1	14
	$lnCO_2$	32		1.22	0.80	0.00	2.64
	t	28		9.5	1.6	7.5	13.2

Table 3

Period	Outcome	Predictor	Equation	R^2
1 (January – May)	pH	q	y = 8.074 (0.222) + 2.918 (0.441) *q - 0.162 (0.045)*t	0.65
		Feed	y = 8.165 (0.077) – 0.020 (0.003) * feed	0.59
	CO_2	q	y = exp(1.075 (0.468) – 7.273 (0.930)*q + 0.285 (0.094)*t)	0.73
		Feed	y = exp(0.263 (0.198) + 0.045 (0.008)*feed)	0.61
2 (June – September)	pH	q	y = 7.464 (0.144) + 2.693 (0.206)*q - 0.086 (0.012)*t	0.90
		Feed	y = 8.304 (0.159) – 0.038 (0.007) * feed	0.50
	CO_2	q	y = exp(3.371 (0.422) – 7.980 (0.600)*q + 0.061 (0.035)*t)	0.87
		feed	y = exp(-0.571 (0.463) + 0.091 (0.022)* feed)	0.40

Captions Tables 1 to 3

Table 1:

Summary of biological data from production of Atlantic salmon in two closed cages (S-CCS) involved in the study, with reference groups in open control cages. The production was split into several time periods because of other technological and biological trials in the same cages during the period from November 2012 to January 2015. Water quality was monitored from 09.01.14 to 25.09.14, with samples from the closed cages and from open cages No. 11 and 14. N_0=number of fish stocked in each cage, N_1=number of fish at closing date, W_0=start weight (g), W_1=final weight, t=number of days, T=average temperature (°C), SR%=Survival in % of stocking number, SGR=Specific growth rate, TGC=Thermal growth coefficient.

Table 2:

Specific water consumption (L/kg/min), feed load per water flow (g/m^3), pH, CO_2 (mg/L), lnCO_2 and temperature (°C) in two closed cages (S-CCS) during two time periods in 2014. Number of samples (n), median values (pH), mean values (all other parameters), standard deviation (SD), minimum and maximum values.

Table 3:

Regression equations of the relationship between pH and CO_2 and q (L/kg/min), feed load per water flow (g/m^3) and temperature (°C). Data from measurements in two S-CCS during two time periods in 2014.

IV

Aquaculture xxx (xxxx) xxx–xxx

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The importance of exercise: Increased water velocity improves growth of Atlantic salmon in closed cages

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ABSTRACT

There is increasing concern about Norwegian salmon farming and the possible environmental impacts from sea lice, escaped fish and release of toxic chemicals and organic emissions to the coastal waters. Closed containment systems (CCS) have the potential to eliminate the problems with sea lice and to reduce escapes and emissions. When closing the cages, water volumes and velocity are regulated and the identification of optimal current velocities for growth and fish welfare from sea transfer to harvest size becomes necessary. This study describes two trials with LOW (0.10–0.27 BL/s) and MODERATE (0.36–0.63 BL/s) water velocity on performance of post-smolt Atlantic salmon in CCS. In trial 1 (168 days, 10.9 °C, fish size: 884–3007 g and 41.5–59.0 cm), round weight increased with 219 g (p = .012) and condition factor with 0.11 (p = .016) in the MODERATE group compared with LOW group. The MODERATE group obtained specific growth rate (SGR) of 0.76 and thermal growth coefficient (TGC) of 2.75, compared to 0.72 and 2.56 in the LOW group. MODERATE water velocity was also associated with higher relative heart size (RHS) (p = .016), higher liver index (HSI) (p = .005), increased fillet yield ($p \leq .001$) and lower levels of cathepsin activity in muscle tissue. In trial 2 (46 days, 7.1 °C, fish size: 327–482 g and 29.9–33.7 cm), round weight increased with 52 g (p = .019) and condition factor with 0.05 (p = .009) in the MODERATE group compared with LOW group. The MODERATE group obtained SGR of 0.77 and TGC of 2.68, compared to SGR of 0.60 and TGC of 2.02 in the LOW group. No significant difference was observed in white muscle cell hyperplasia, measured as the proportion of small (< 20 µm diameter) muscle fibres (p = .145). Both trials showed only minor differences in slaughter yield, fillet quality (protein, fat, water) and mortality. The present study shows that moderate water velocity (0.36–0.63 BL/s) is favourable for growth rates for Atlantic salmon during the entire on-growing period in CCS. Effects on a broader range of metabolic variables and welfare indicators were also documented.

1. Introduction

Production of post-smolt Atlantic salmon (*Salmo salar*) primarily occurs in netpen cages in coastal areas. With Norwegian salmon farming's rapid growth in the last few decades, the environmental impact of salmon production has received more public attention. Negative effects on wild salmon populations by spread of diseases (Garseth et al., 2013) and escaped fish (Naylor et al., 2000; Naylor et al., 2005) and the potential negative effects of nutrient overloading in the coastal areas are important controversies to solve if Norwegian aquaculture should continue to grow. Problems with salmon lice (*L.salmonis*) on both farmed salmon and wild salmonids and the emergence and rapid spread of drug-resistant lice have forced farms to abandon chemical treatments and to develop non-medicinal treatments or alternative farming strategies (Hjeltnes et al., 2018).

One possible solution to prevent salmon lice infestation in salmon farms is to use more closed cage technologies. In closed containment systems (CCS) (Calabrese, 2017) intake water can be pumped from deeper water layers avoiding infective salmon lice copepodites (Nilsen et al., 2017a). Using a rigid, closed cage design or tarpaulin bags with surrounding safety nets and using sites better sheltered from extreme wind and waves could reduce the risk of escaped fish. In addition, the local environmental impact can be reduced by collecting and reusing settleable particles from faeces and surplus feed.

Water velocity is an environmental parameter with a profound impact on fish metabolism, growth, behaviour and welfare (Palstra and Planas, 2011). First of all, higher water velocity can boost the growth of farmed fish (Leon, 1986; Christiansen et al., 1989; Jobling et al., 1993; Jørgensen and Jobling, 1993; Young and Cech, 1993; Davison, 1997;

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Castro et al., 2011; Li et al., 2016; Ytrestøyl et al., 2017). Higher growth rates have been linked to increased feed intake and more effective feed-conversion ratio (Jobling et al., 1993; Davison, 1997; Castro et al., 2011). Higher water velocity also improves flesh texture (Totland et al., 1987; Tachibana et al., 1988; Bugeon et al., 2003; Li et al., 2016) and general robustness (Takle et al., 2010) and may lower aggression (Kalleberg, 1958; Jobling et al.; 1993; Solstorm et al., 2016) and lead to a reduced stress response (Woodward and Smith, 1985; Young and Cech 1993; Huntingford, 2010; Solstorm et al., 2015). On the other hand, too high water velocities will lead to increased oxygen need and anaerobic metabolism with increased levels of lactate (Davison, 1997; Palstra et al., 2010) and finally to exhaustion, reduced growth and impaired fish welfare (Solstorm et al., 2015; Solstorm et al., 2016).

CCS operation requires pumping large volumes of water with a continuous oxygen supply (Nilsen et al., 2017b; Summerfelt et al., 2016; Sveen et al., 2016). With intensified CCS production, reduced specific water consumption (SWC) could reduce both water quality and water velocity. If the water is oxygenated but not aerated, the build-up of fish metabolites, especially carbon dioxide (CO_2) and total ammonia nitrogen (TAN) will set the limit for lowest acceptable water flow (Sanni and Forsberg, 1996; Bergheim and Fivelstad, 2014). Regulating CCS water flow facilitates the regulation of water velocity to optimise fish growth and welfare. Most studies on different water velocities have been performed over shorter periods with small fish in freshwater. While there are some studies on how salmon adapt to different water velocities in open sea cages (Oppedal et al., 2011; Johansson et al., 2014), there is a need for more specific knowledge about the effect of continuous water velocity in CCS on growth, flesh quality and welfare in Atlantic salmon grown from post-smolt to harvest size.

The present study's main aim was to investigate how two different water velocities on post-smolt Atlantic salmon in a CCS affected growth, chemical composition of fillet, myotomal cathepsin activity and muscle cell development. We also observed fish behaviour and evaluated the results from a fish welfare perspective.

2. Materials and methods

2.1. Cages and water quality

Circular CCS of 40 m^3 volume were used in the present experiment (AkvaDesign AS, Brønnøysund, Norway). The CCS consisted of tarpaulin bags with water pumped from 25 m depth (Nilsen et al., 2017a). Water was pumped (5.5 kW, Xylem Norway AS) into a floating tank (4.5m^3) and then distributed to each cage securing identical temperature and quality of inlet water to all cages. Water flow was 250–275 L/min for each cage, with retention time of approximately 2.5 h. The current in the three LOW velocity cages (6–8 cm/s) was created by incoming water alone. In the three MODERATE velocity cages (19–21 cm/s), an extra current booster (D-Icer 1, 6228D 2HPw, Taylor Made Products, 65 Harrison street, Gloversville, NY 12078) was located opposite to the water inlet pipe. The current boosters were installed at 1 m depth, 0.5–0.75 m from the cage wall (Fig. 1). Water velocity was kept constant throughout the experiment and, consequently, the relative swimming velocity (BL/s) decreased as the fish grew.

The cages were circular with a diameter of 4.8 m and a total depth of 4 m (ratio of 1:1.2), with a short 60° open-ended inlet located at 0.5 m depth creating a circular, primary current, see Fig. 1. Each cage was supplied with external light mounted on the floating ring supporting the tarpaulin bags (LED 2x50W 230 V IP65, Etman Distribusjon AS, Egersund, Norway). Oxygen was supplied by a diffusor net (AkvaDesign AS), oxygen and temperature were logged with 10-min intervals at 2 m depth (FDO 700 IQ SW, WTW/Xylem). Mean oxygen saturation was regulated to 80–95% in all cages. Water quality parameters such as pH, temperature, dissolved oxygen and salinity were measured with SmarTroll MP handheld sensor (Tormatic AS, Norway). Carbon dioxide was measured with OxyGuard CO_2 portable meter (OxyGuard AS, Denmark). Water samples for laboratory analysis of pH (NS-EN ISO 10523), total ammonia nitrogen (TAN, NS-EN ISO 14911) and total suspended solids (TSS, NS-EN 872) were collected at 1 m depth with Ruttner type water sampler (Fybikon AS, Norway), and stored at + 4 °C in sterile plastic bottles for chemical water analysis until delivery to the laboratory (Kystlab Prebio, Brønnøysund, Norway).

2.2. Fish and rearing conditions

Trial 1 lasted from June to November 2015, and trial 2 from February to April 2017. The fish were fed to satiation every day, using Betten feeders S1–125 automatic feeding system (Betten Maskinstasjon AS, Vågland, Norway). All experimental procedures were in accordance with the regulations controlling experiments/procedures on live animals in Norway, and the study complies with the policies relating to animal ethics. Due to the experiment's nature, permission from the Norwegian Research Authority was not required. All fish were returned to one of the commercial cages after the experiment.

Dead fish were collected two to five times per week with the lift-up integrated in the water outlet. Injured or weak fish were netted, killed and recorded as mortality. All dead or killed fish were inspected, weighed and autopsied. Kidneys were examined for macroscopic signs of nephrocalcinosis, a typical lesion when levels of CO_2 exceeds 10–15 mg/L (Fivelstad et al., 2003; Fivelstad et al., 2018) and gills were examined for macroscopic signs of gill diseases. Cause-specific mortality was scored in five categories: (1) ulcers and fin lesions, (2) physical trauma, (3) infectious diseases, (4) runts or (5) unknown.

Trial 1: Atlantic salmon (*S. salar*, Salmo breed) from Bindalssmolt AS, 7982 Bindalseidet, Norway. From sea transfer in November 2014 until May 2015 the fish were reared in commercial-scale CCS (2870 m^3 volume) at site Møllebogen (65°N 12° E), and then moved to Norsk Havbrukssenter, Brønnøysund, Norway (65.5°N 12.1°E) where the trial took place from June 10th 2015 to November 17th 2015. On May 27th 2015, 1800 salmon (mean ± SE weight 894 ± 4.6 g, length 41.5 ± 0.06 cm) were evenly distributed into the six closed cages. Each cage was stocked with 300 salmon, 250 fish untagged and 50 fish PIT tagged intraperitoneally with GPT12 Pre-load tags (12.5 mm, 134.2 kHz) (Biomark, Boise, USA), using MK25™ Implant Gun (Biomark, Boise, USA). Biomark 601™ Reader (Biomark, Boise, USA) was used to read the PIT tag ID. Water velocity was adjusted to LOW (6 ± 0.4 cm/s) and MODERATE (21 ± 0.7 cm/s) June 10th. This corresponded to an initial water velocity of 0.14 and 0.5 body lengths per second (BL/s) and final water velocity of 0.10 and 0.36 BL/s respectively. The last feeding day was November 15th with final sampling on November 17th and 18th. During the trial, one cage in each group was excluded due to large variations in oxygen levels and/or water velocity, and the presented data are therefore based on the four remaining cages. Feeding was Spirit S600-50A 7 mm from 10.6 to 29.7, Premium 1200-50A 9 mm from 30.7–14.10, and Premium 2500-50A 9 mm from 15.10 to 17.11 (Skretting AS, Stavanger, Norway). Stocking density was between 7 kg/m^3 and 20 kg/m^3. The trial lasted for 168 days.

Trial 2: Atlantic salmon (*S. salar*, AquaGen) from Grytåga settefisk, 8860 Tjøtta, Norway. From sea transfer in October 2016 until January 2017 the fish were reared in commercial scale CCS (6000 m^3 volume) at site Sæterosen (65.3°N 12.3°E), AkvaFuture AS, 8900 Brønnøysund, Norway. On January 31st, 7200 post-smolt salmon with an average weight of 300 g were evenly distributed into six closed cages. After one month with acclimatisation to low speed in all cages, mean weight and length in each cage were determined on February 27th by randomised sampling of 150 fish from each cage. Water velocity was adjusted to LOW (8 ± 0.6 cm/s) and MODERATE (19 ± 0.7 cm/s), corresponding to initial water velocity of 0.27 and 0.63 BL/s and final water velocity of 0.24 and 0.57 BL/s respectively. The trial started on March 1st 2017. The last feeding day was April 17th, the trial ended on April 18th, and the final samples were taken April 19th to 20th. Feeding was Intro 200 HH 50 mg Q 5 mm (Biomar AS, Myre, Norway). Stocking density was

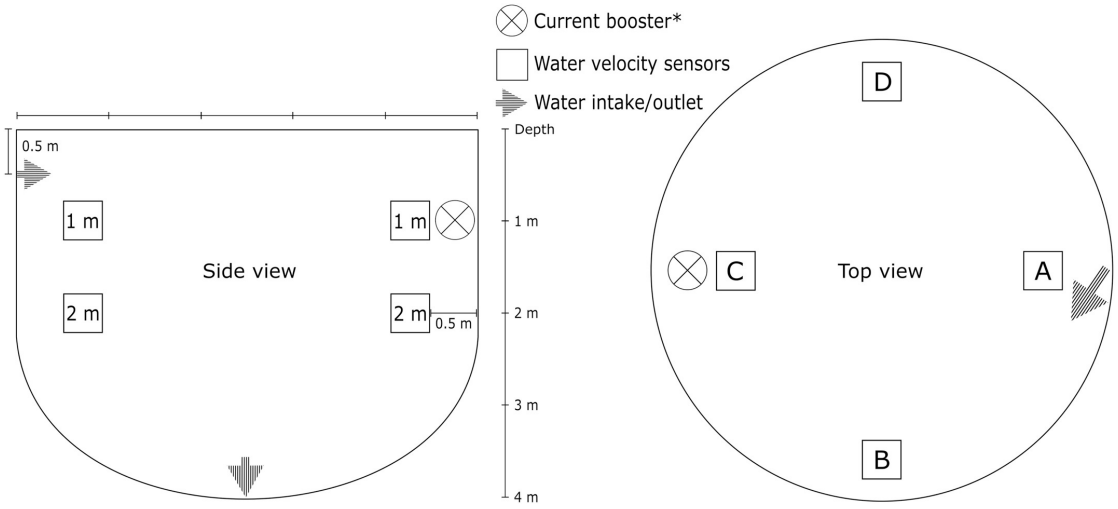

Fig. 1. Schematic presentation of the test cages. Left: side view with location of inlet, outlet, current booster (* only in the MODERATE group) and the positions where water velocity was measured. Right: top view with location of water inlet, current booster and the positions where water velocity was measured.

between 10 kg/m^3 and 13 kg/m^3 and the trial lasted for 46 days.

2.3. Water velocity and fish behaviour

Trial 1: The circular, horizontal water velocity was measured weekly (1 to 3 min' duration in each position, continuous logging) with Flow Rate Sensor LQ2-LE with the software Labquest 2 (Vernier Software and Technology, USA, www.Vernier.com). These measurements were validated with an SD6000 current sensor (Nortek AS) in October 2015. Three parallel measures in LOW and MODERATE cages with Vernier Flow Rate sensor and Nortek SD6000 showed no significant difference between the two methods, and the results from these methods in trial 1 are reported in the same tables and figures. Water velocity was measured at position A, C and D (1 and 2 m depth, 0.5 to 0.75 m from the cage wall, see Fig. 1), and reported at group level as mean velocity (SE) of all positions and both depths from the two remaining cages in each group. Careful observations of the fish were conducted once a week on the presence of individuals with visible lesions and on behaviour: schooling behaviour, vertical positioning, abnormal behaviours, feeding activity and swimming speed relative to water velocity.

Trial 2: Water velocity was measured weekly (0.1 to 70.4 h duration in each position, 10 min logging intervals) with two SD6000 current sensors (Nortek AS) at 1 and 2 m depths in positions A and C (Fig. 1), and reported at group level as mean velocity (SE) of both positions and depths from all three cages in each group. Fish behaviour and lesions were observed as in trial 1.

2.4. Sampling procedures

Trial 1: Weight and length of all fish were recorded on May 27th, of 50 fish in each cage on August 26th, and of 200 fish in each cage on November 17th. The fish were collected by purse seine and taken using scoop net and anesthetised with tricaine methanesulfonate (Tricaine Pharmaq 1000 mg/L, Pharmaq AS) 30–80 mg/L, before measuring. At each of the three sampling dates, 15 untagged fish from each cage were randomly sampled for the evaluation of biometrics and chemical fillet analysis. This fish were stunned by a sharp blow to the head, gill arches on both sides were cut and the fish bled for half an hour in cold seawater and then individually labelled. Sampling at the site included round weight, fork length, gutted weight, liver weight and heart weight (including the bulbus arteriosus) of each individual. The fish were then iced in polystyrene boxes and sent by boat to the Faculty of Biosciences and Aquaculture, Nord University (Bodø, Norway) and stored at +4 °C awaiting further analysis of fillet chemical content and analysis of Cathepsin B, B + L and H.

Trial 2: Weight and length of 100 fish in each cage were recorded on February 27th and 150 fish in each cage on April 18th. Fish were anaesthetised with benzocaine (Benzoac Vet 200 mg/mL, ACD Pharmaceuticals AS) 0.2–0.25 mL/L. Sampling included the same parameters and procedures as in trial 1, except from the enzyme analysis and with additional sampling for muscle cell analysis.

2.5. Biometrics

Weight (W) was recorded as round body weight in g (± 1 g), length (L) as fork length (± 0.5 cm) and reported as group mean and standard deviation (SD). Condition factor was calculated as:

$$CF = 100 \cdot (W/L^3)$$

Specific growth rate (SGR) was calculated as (Houde and Scheckter, 1981):

$$SGR = 100 \cdot (\ln(W_1) - \ln(W_0))/(t_1 - t_0)$$

where W_1 and W_0 are weights on days t_1 and t_0, respectively. Thermal growth coefficient (TGC) was calculated as (Alanärä et al., 1994):

$$TGC = 1000 \cdot (W_1^{1/3} - W_0^{1/3})/(T \cdot t)$$

where T is temperature in °C and t is time in days.

The SGR and TGC models are limited to fish size between 50 and 3000 g and temperatures between 4 °C and 14 °C (Alanärä et al., 2001).

Slaughter yield was calculated as: 100·(gutted weight/round body weight). Fillet yield was calculated as: 100·(weight of both fillets/round body weight). Hepasomatic index (HSI) was calculated as: 100·(liver weight/ round body weight). Relative heart size (RHS) was calculated as: 100·(heart weight/round body weight). Water velocity relative to fish size was calculated as: body lengths per second (BL/s). Production intensity in the cages was calculated as: specific water consumption (SWC) in litres of water used per kg fish per minute (L/kg/min), feed load (FL) in g feed/m^3 water flow (g/m^3) and density as kg fish per m^3

cage volume (kg/m^3).

2.6. Fillet chemical composition and cathepsin activity

At day 5 post mortem, the fish was filleted and both fillets were weighed. The Norwegian quality cut (NQC) (NS 9401, 1994) from the right fillet was homogenised in a Braun MR530 Turbo-Accesorios homogeniser (Braun, Germany), and the mince stored at −40 °C before chemical analysis (both trials) of protein, fat, water content, and cathepsin (only trial 1) B, B + L and H activity, in duplicates. In trial 1, all homogenised samples from sample one were analysed with the following chemical reference methods: water content was determined after drying at 104 °C for 20 h, protein analysed as Kjeldahl-nitrogen using factor 6.25 (Kjeltec 1030 Auto analyzer; Foss Tecator AB, Höganäs, Sweden), and fat was determined by extraction in ethyl acetate (Norwegian Standard NS-9402E, 1994E). In sample two and three, a representative sample of 50% of the fish were analysed with chemical methods and all homogenised samples were consecutively scanned by Near Infrared Spectroscopy (NIR) before freezing, using DA 7200 Diode Array High Speed analyzer (Perten Instruments AB, Hägersten, Sweden). The instrument was operating in the wavelength range of 950–1650 nm. In trial 2, all samples were scanned by NIR and 50% of the samples were analysed with chemical methods. A representative selection of fish from the last sampling in trial 2 were used for calibration of NIR data to chemical analysed data of water (six factors, $R^2 = 0.984$), fat (five factors, $R^2 = 0.978$) and protein (nine factors, $R^2 = 0.976$) respectively, using PLS regression in the Unscrambler® X software (version 10.4, CAMO Software AS, Oslo, Norway). The PLS models were configured with full cross-validation and SD-1 weighting of the Y-variable, further used for prediction of individual content of water, protein and fat for all fish in trail 2, according to previously described methods (Solberg, 1992, 1997). Cathepsin B, B + L and H acitivity (trial 1) were analysed as described by Hagen et al. (2008).

2.7. Muscle cell analysis

In trial 2, two fish from each cage at initial sampling, and five fish from each cage at final sampling were analysed for muscle cell histology according to Johnston et al. (1999). A 5 mm thick cross-sectional slice was taken directly behind the dorsal fin, and three muscle blocks (0.5 × 0.5 × 0.5 cm) were cut out (Fig. 2) covered with Shandon™ Cryomatrix™ (Thermo Fisher Scientific, MA, USA) and frozen in isopentane cooled in liquid nitrogen (−159 °C) for 60 s, wrapped in aluminium foil and stored at −80 °C until preparation. Transverse muscle sections were cut at 8 µm in a cryostat (CryoStar NX50, Thermo Fisher Scientific, MA, USA) and stained with hematoxylin solution (Papanicolaou's solution 1a Harris' hematoxylin solution, Merck, Germany). Slides were examined with light microscopy (Axioskop 2 mot plus, Zeiss, Germany) and photographed with a digital camera (Axiocam HRc, Zeiss, Germany) mounted directly on the microscope with 10× magnification. Using Axiovision 4.8 (Zeiss, Germany) the circumference of a minimum of 450 white muscle cells (fast cells) were measured for each fish, and cell density, diameter and cell area was calculated.

2.8. Statistical analysis

Weight, length, condition factor and all qualitative outcome variables are reported as group means with standard deviation (SD). Statistical analysis of the effect of water velocity on growth data, chemical content and cathepsin activity were performed using the IBM SPSS Statistics v. 22.0 (IBM Corporation, NY, US). Weight, length, condition factor (CF), RHS, HSI, slaughter yield and fillet yield were analysed with a mixed linear regression model (maximum likelihood) with group as fixed effect and cage as random effect. The effects of water velocity on chemical analysis and enzyme activity were analysed with group as fixed effect and both cage and gutted weight as random effects. The effect of gutted weight on the model was from low to moderate and the results from the analysis with both cage and gutted weight as random effects are reported. Residuals were plotted with a P–P plot, the effect of extreme outliers on the models was evaluated and if necessary they were removed before the final analysis. The statistical analysis is reported as the differences between the MODERATE and LOW groups with 95% confidence intervals and *p*-values.

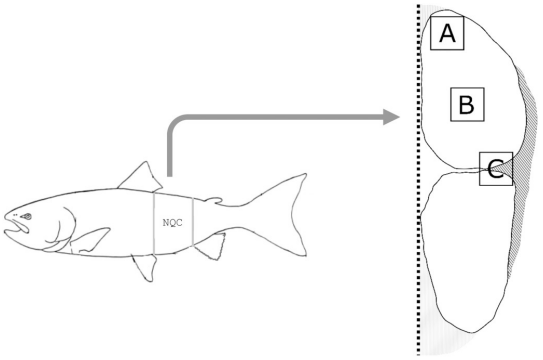

Fig. 2. Schematic view of Norwegian quality cut (left), and the sample sites for muscle fibre analysis (right). The right panel shows the 5 mm thick cross-sectional slice of the fish, taken directly behind the dorsal fin, with the location of three muscle blocks from each fish: A and B from epaxial white muscle fibres, C from white and red muscle fibres at the lateral line.

The muscle cell distribution was analysed using R (3.3.1). Distribution of muscle cell diameter was evaluated using smooth non-parametric distributions where 450 measurements of cell diameter were fitted using a kernel function (Johnston et al., 1999). Groups compared had similar body mass and length (n = 9 LOW group, n = 13 MODERATE group). A Kolmogorov–Smirnov two-sample test was used to test the null hypothesis that the probability density functions (PDFs) of groups were equal over all diameters. Density curves for each treatment were also compared graphically by constructing a variability band around the density estimate for the combined populations using the mean smoothing parameter h, varying between 0.17 and 0.19 for the different groups (Bowman and Azzalini, 2003). This can be used to distinguish the underlying structure in the distributions from random variation providing an indicator of which part(s) of the distribution of diameters contributed to any significant differences.

3. Results

3.1. Growth

The mean weight and condition factor was higher in the MODERATE velocity group at the end of both trials (Tables 1 and 4, Fig. 3). In trial 1, the MODERATE group had 7.9% increased weight, in trial 2, 12.1% increased weight compared with LOW. There were no differences in start weight, length and condition factor between the two groups. At mid-evaluation (trial 1) the groups were also equal.

In trial 1, SGR increased by 5.9% from LOW (0.68) to MODERATE (0.72), TGC increased by 7.4% from LOW (2.56) to MODERATE (2.75). In the individually tagged fish in trial 1 (total n = 120), mean SGR (SD) in the LOW group was 0.63 (0.13), in the MODERATE group 0.67 (0.10), an increase of 6.3%. In trial 2 SGR increased with 28% from LOW (0.60) to MODERATE (0.77), and TGC with 33% from LOW (2.02) to MODERATE (2.68) (Table 2).

Table 1
Number of sampled fish (n), mean (SD) weight (g), length (cm) and condition factor (CF) in Atlantic salmon exposed for either LOW or MODERATE water velocities in two separate trials (168 days in trial 1, 46 days in trial 2).

Trial		Sample	LOW			MODERATE		
			n	Mean	SD	n	Mean	SD
1	Weight (g)	1	600	884	112	600	894	115
		2	100	1392	279	92	1435	252
		3	425	2782*	546	438	3003*	548
	Length (cm)	1	600	41.5	1.6	600	41.7	1.6
		2	100	48.7	2.7	98	49.1	2.3
		3	425	58.9	3.7	438	58.9	3.6
	CF	1	599	1.24	0.08	600	1.23	0.07
		2	100	1.19	0.10	89	1.21	0.10
		3	425	1.34*	0.12	433	1.45*	0.13
2	Weight (g)	1	292	327	111	296	338	127
		2	449	430*	161	447	482*	194
	Length (cm)	1	292	29.9	3.5	296	30.2	4.0
		2	449	33.0	3.9	447	33.7	4.2
	CF	1	292	1.19	0.09	296	1.17	0.10
		2	449	1.14**	0.08	447	1.19**	0.08

Significant differences between groups are indicated with: *:$p \leq .05$, **:$p \leq .01$.

3.2. Biometry

In trial 1, moderate water velocity increased the relative heart size (RHS) with 0.008%, the liver index (HIS) with 0.05% and fillet yield with 1.96% (Table 4). In trial 2, there were no differences in RHS or HSI and fillet yield was not recorded.

3.3. Fillet chemical content and cathepsin activity

There were only small differences in chemical composition of fillets in both trials (Table 3, Table 4). Fat (and thus also water) content was highly affected by body weight and using both cage and gutted body weight as random effects in the linear regression model removed most of the effect of water velocity on water, fat and protein content.

Cathepsin levels in both groups (only trial 1) decreased during the trial (Table 3). At the end of trial 1, mean activity levels of all three cathepsins (B, B + L, H) were lower in the MODERATE group compared with LOW when analysed with cage and gutted weight as random effects, but with a significant level of effect only in cathepsin B ($p = .008$) and cathepsin H ($p = .044$). (Table 4).

3.4. Muscle cellularity

At the end of trial 2, white muscle hyperplasia measured as the proportion of small fibres (< 20 µm diameter) showed no significant difference between MODERATE and LOW. The overall fibre distribution after removing 11 outliers due to extreme size or extremely different fibre diameter distribution did show a significant difference between groups (Kruskal-Wallis, $p = .005$), but this was not consistent with the probability density distribution in Fig. 4 which shows no deviation from the probability density area.

3.5. Water velocity and fish behaviour

The mean (SE) measured horizontal water velocity in trial 1 was 6 cm/s (0.4) in the LOW group and 21 cm/s (0.7) in the MODERATE group. In trial 2, water velocity was 8 cm/s (0.6) and 19 cm/s (0.7), see Table 5. The formation of a free vortex or irrotational zone with poorer mixing or lower velocities close to the centre drain, as described by Timmons et al. (1998), was not observed. The majority of observations in all cages and both trials showed a schooling behaviour where the fish swam counter-current with swimming speed slightly faster than the water velocity, and most (typically > 90%) fish formed a "doughnut" distribution at 0.5 to 2.5 m depth, with detours to the surface during feeding cycles and as part of the usual rolling and jumping behaviour and to refill the swim bladder. If current velocity dropped below 2 cm/s (LOW group) the schooling activity tended to disintegrate, with fish starting to swim in all directions. Immediately after transfer to the research cages, we observed fish with loss of scales in all cages. Some of these developed into skin lesions and ulcers, as described under 3.7 Mortality.

3.6. Temperature and water quality

The temperature profiles for both trials are shown in Table 6. The mean oxygen levels in all cages were between 82.8 and 94.7% DO (Table 7). The measured level of CO_2 in trial 1 was ≤ 2 mg/L. In both trials pH was between 7.4 and 7.9 in all cages, corresponding to CO_2 levels < 8 mg/L (Nilsen et al., 2017b). Total ammonia Nitrogen (TAN) values were ≤ 0.7 mg/L, with salinity 32.0 ppm and pH ≥ 7.4 corresponding to levels of toxic ammonia (NH_3) < 0.004 mg/L (Fivelstad et al., 1995). Levels of suspended solids (TSS) were < 20 mg/L. The specific water consumption (L/kg/min) was, in trial 1, between 0.31 and 0.94, and, in trial 2, between 0.31 and 0.42. The feed load (g/m^3) in trial 1 was between 6.1 and 17.4, and, in trial 2, between 11.3 and 15 (Table 8).

3.7. Mortality

Mortality in trial 1 was 7.7% in the LOW group and 6.5% in MODERATE group (Table 9). Cause-specific mortality was classified as «Unknown» (58%), «Ulcer and fin lesions» (32%) and «Other trauma» (10%). Mortality in trial 2 was 1.9% in LOW group and 2.3% in MODERATE group. Cause-specific mortality was classified as «Ulcer and fin lesions» (91%), «Runts» (7%) and «Unknown» (2%). No signs of other infectious diseases, gill lesions or kidney lesions (nephrocalcinosis) were detected.

4. Discussion

The trials' aim was to determine the effect of two different water velocities on growth, muscle cellularity, chemical composition and enzyme cathepsin activity of muscle in post-smolt Atlantic salmon in closed cages (CCS). The main finding in both trials is enhanced growth with increased water velocity.

4.1. Growth and muscle cell hyperplasia

In the MODERATE group (0.4–0.6 BL/s), the salmon had 7.9–12.1% increased weight (1.1–1.3 g/day) compared with the LOW group (0.1–0.3 BL/s). The relationship between growth and water velocity is demonstrated in several studies covering salmonids and other farmed species (Leon, 1986; Totland et al., 1987; Christiansen et al., 1989; Jørgensen and Jobling, 1993; Martin and Johnston, 2005; Palstra and Planas, 2011; Davison and Herbert, 2014). Other studies also show minor differences in length with increasing water velocity (Martin and Johnston, 2005; Davison and Herbert, 2014). In one of the few studies on large salmon in seawater, Totland et al. (1987) compared adult salmon (2 kg) in a swimming raceway for 8 months at 0.45–0.40 BL/s with fish in standard cages, with 38% higher weight gain in the raceway system. The durations of our trials were 168 and 46 days. Further studies with longer trial periods on large fish, and trials in commercial scale cages are necessary to establish the optimal water velocity for growth and fish welfare in CCS.

The SGR in our trials were between 0.60 and 0.77 and lower than common industry standards. Expected SGR for salmon of 300–500 g at 6–7 °C is 0.77–1.06 and 0.49–1.31 for salmon of 800–3000 g at 9–14 °C (Skretting, 2012). The TGC between 2.02 and 2.68 was also lower than

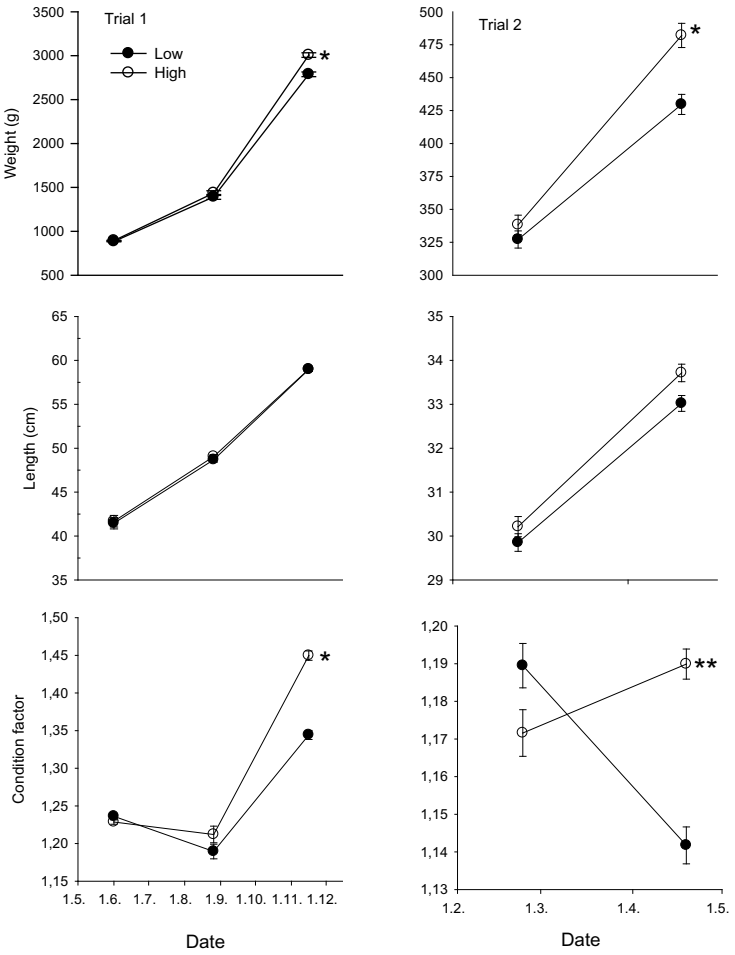

Fig. 3. Mean (SE) weight (g), length (cm) and condition factor in Atlantic salmon exposed for either LOW or MODERATE water velocities in two separate trials (168 days in trial 1, 46 days in trial 2). Significant differences between groups are indicated with: *: $p \leq .05$, **: $p \leq .01$.

Table 2
Number of sampled fish (n), thermal growth coefficient (TGC) and specific growth rate (SGR) in Atlantic salmon exposed for either LOW or MODERATE water velocities in two separate trials (168 days in trial 1, 46 days in trial 2). Data from total samples of fish in both trials, and from an individually tagged subsample of fish (mean and SE) in trial 1.

		LOW			MODERATE		
		n	TGC	SGR	n	TGC	SGR
Trial 1	Total	425	2.56	0.72	438	2.75	0.76
	Tagged	52		0.63 (0.13)	68		0.67 (0.10)
Trial 2	Total	449	2.02	0.60	427	2.68	0.77

expected growth rates (2.7–3.0) in commercial closed cages (Thorarensen and Farrell, 2011; Nilsen et al., 2017a). These moderate growth rates could be caused by stress from handling procedures prior to the experiment and the environmental change caused by transfer to smaller research cages. Longer periods of acclimatisation could have improved the growth during the trials. Growth, measured as mean body weight and CF, stagnated for both groups at mid-sampling in trial 1. In trial 2, the condition factor in the LOW group was even reduced from 1.19 to 1.14 during a trial period of 46 days. Furthermore, TGC of 2.02 in the LOW group in trial 2 indicates suppressed growth in this group. In trial 1, two cages were excluded, and the study's statistical strength reduced. Nevertheless, the group differences in weight and condition factor, after adjusting for cage effects, were significant in both trials. The differences in growth rates between the LOW and MODERATE groups of tagged fish and total population in trial 1 were similar to the total group data, supporting the overall results. The reduced SGR in the tagged fish compared to the whole group could have been caused by adverse reactions to the tagging.

The increased growth is principally muscle growth, as the salmon in the MODERATE group in both trials had increased weight, but no or

Table 3
Number of sampled fish (n), mean (SD) relative heart size (RHS), hepatosomatic index (HSI), slaughter yield (% of round body weight), fillet yield (% of round body weight, only trial 1), fillet content of protein, fat and water (% of total fillet weight) and activity of cathepsin enzymes (cathepsin B, B + L and H, only trial 1, measured as mmol AMC/min/g) in muscle tissue in Atlantic salmon exposed to either LOW or MODERATE water velocity (168 days in trial 1, 46 days in trial 2).

Trial	Parameter	Start			LOW			MODERATE		
		n	Mean	SD	n	Mean	SD	n	Mean	SD
1	Relative heart size (RHS %)	53	0.10	0.02	30	0.11*	0.01	30	0.12*	0.01
	Liver index (HSI %)	53	0.86	0.28	30	0.99**	0.07	30	1.04**	0.06
	Fillet yield (%)	53	66.8	3.2	30	64.1***	1.5	30	66.0***	2.0
	Slaughter yield (%)	53	90.2	1.5	30	87.9	1.2	30	88.1	1.0
	Water (%)	60	69.0	1.2	30	65.0	1.1	30	64.3	1.1
	Fat (%)	60	10.5	1.3	30	15.7	1.6	30	16.2	1.3
	Protein (%)	59	20.2	0.4	30	19.1	0.4	30	18.9	0.4
	Cat B (mmol AMC/min/g)	60	1077.1	192.4	30	898.2**	204.8	30	679.8**	130.4
	Cat B + L (mmol AMC/min/g)	60	562.2	109.8	30	414.2	90.0	30	339.6	80.7
	Cat H (mmol AMC/min/g)	60	340.1	147.4	30	278.9*	125.6	30	181.4*	119.9
2	Relative heart size (RHS %)	59	0.11	0.01	23	0.11	0.02	30	0.11	0.01
	Liver index (HSI %)	58	1.13	0.21	25	1.00	0.11	30	1.08	0.25
	Slaughter yield (%)	58	84.6	2.5	24	88.5	1.4	30	87.3	1.8
	Water (%)	60	68.0	1.2	30	69.6*	1.6	30	68.6*	1.4
	Fat (%)	60	10.9	1.5	30	9.4	1.8	30	10.4	1.8
	Protein (%)	60	19.1	0.8	30	20.5**	0.2	30	20.3**	0.2

Table 4
The effect of MODERATE water velocity compared with LOW water velocity on growth, fish quality and cathepsin enzyme activity (mmol AMC/min/g) analysed with a mixed model ML linear regression. The differences between MODERATE and LOW velocity groups are reported with 95% confidence intervals and p-values.

Trial	Parameter	Effect of MODERATE water velocity			
		Diff.	Low 95% CI	High 95% CI	p-Value
1	Weight (g)	219	79	358	0.012*
	Condition factor (CF)	0.11	0.03	0.18	0.016*
	Relative heart size (%)	0.008	0.001	0.014	0.016*
	Liver index (%)	0.05	0.02	0.08	0.005**
	Fillet yield (%)	1.96	1.06	2.86	< 0.001***
	Cat B (mmol AMC/min/g)	−218.4	−342.1	−94.7	0.008**
	Cat B + L (mmol AMC/min/g)	−77.7	−176.5	21.1	0.094
	Cat H (mmol AMC/min/g)	−97.6	−191.4	−4.3	0.044*
2	Weight (g)	52	12	92	0.019*
	Condition factor (CF)	0.05	0.02	0.08	0.009**
	Water (%)	−0.8	−1.4	−0.1	0.030*
	Protein (%)	−0.16	−0.28	−0.04	0.009**

Table 5
Water velocity as cm/s (mean and SE). Relative water velocity as body lengths per second (BL/s) is reported from the start (BL/s_0) and end (BL/s_1) of each trial. Current velocity was measured at 1 and 2 m depth, during 15.07.15 to 16.11.2015 (trial 1) and 28.02.2017 to 10.04.2017 (trial 2).

	LOW				MODERATE			
	cm/s	SE	BL/s_0	BL/s_1	cm/s	SE	BL/s_0	BL/s_1
Trial 1	6	0.4	0.14	0.10	21	0.7	0.50	0.36
Trial 2	8	0.6	0.27	0.24	19	0.7	0.63	0.57

Table 6
Trial length in days and temperature (°C) mean, maximum and minimum. Trial 1: June to November 2015, trial 2: March to April 2017. In each trial, the temperatures were identical in all cages.

	Days	T (°C)		
		Mean	Min	Max
Trial 1	168	10.9	8.7	14.2
Trial 2	46	7.1	6.2	7.6

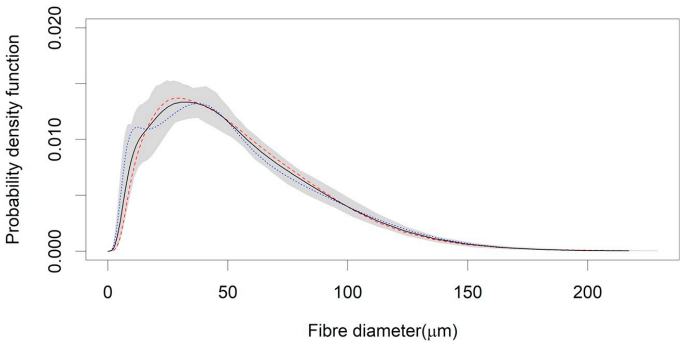

Fig. 4. White muscle fibre distribution in Atlantic salmon exposed for either LOW or MODERATE water velocities (Trial 2). Plot of distribution of fibre size data in μm. Dotted line: LOW group, dashed line: MODERATE group, solid line: total mean and with the 95% probability distribution as the grey area. Samples from 5 fish in each cage, three muscle samples from each fish, a minimum of 150 fibres from each muscle sample.

Table 7
Levels of dissolved oxygen (DO %) in all cages in trial 1 and trial 2; mean, SD, minimum and maximum levels, excluding data from Trial 2, cage L1, in the period from 09.03 to 12.03.

	DO %	LOW			MODERATE		
		L1	L2	L3	M1	M2	M3
Trial 1	Mean	93.8	95.8		94.0	87.6	
	SD	16.2	16.5		9.8	2.7	
	Min	72.3	85.2		82.6	85.3	
	Max	131.7	139.9		113.3	93.2	
Trial 2	Mean	94.7	85.1	82.8	83.1	83.0	83.9
	SD	4.9	4.5	2.3	2.0	2.2	2.7
	Min	82.3	76.7	78.9	79.9	79.4	78.7
	Max	126.6	113.8	106.0	93.6	88.4	143.5

Table 8
SWC = specific water consumption (L/kg/min), FL = feed load (g feed/m^3 water) at start and end of each trial. Density was between 7 and 20 kg/m^3 in trial 1, between 10 and 13 kg/m^3 in trial 2.

	SWC (L/kg/min)		FL (g/m^3)	
	Start	End	Start	End
Trial 1	0.94	0.31	6.1	17.4
Trial 2	0.42	0.31	11.3	15

Table 9
Number of fish (cages) in each group, total accumulated mortality (CM_{total}%) at cage level in LOW and MODERATE groups.

Trial	n	LOW				MODERATE		
		Days	CM_{total}%	Min	Max	CM_{total}%	Min	Max
1	600 (2)	168	7.7	6.0	9.3	6.5	5.0	8.0
2	3600 (3)	46	1.9	1.4	2.9	2.3	1.4	2.8

small differences in length compared to the LOW group. Thus, the condition factor increases with water velocity. This agrees with other studies on Atlantic salmon (Totland et al., 1987; Kiessling et al., 1994; Castro et al., 2011; Solstorm et al., 2015). Histology of muscle in trial 2 showed no clear effect on white fibres. Other studies have shown that higher swimming speed increases the size of white muscle cells (Greer Walker, 1971; Greer Walker and Pull, 1973; Davison and Goldspink, 1977; Davison and Goldspink, 1978). It is possible that the difference in water velocity was too small and the test period too short to produce significant differences between the groups.

4.2. Biometry, fillet chemical composition and cathepsin activity

Increased relative liver and heart size in trial 1 indicates increased metabolism and improved cardiac output in the MODERATE group. This is supported by previous studies on both pre-smolt (Castro et al., 2011) and post-smolt Atlantic salmon (Solstorm et al., 2015). Higher swimming speed is also shown to increase the stroke volume, cardiac output and maximum power output in rainbow trout (Farrell et al., 1991). Increased HSI might reflect an enhanced metabolic activity normally associated with increased nutrient utilisation (Jobling, 1985). The difference in fillet yield in trial 1 is most likely an effect of a larger fish with increased CF.

Differences in body composition (% of water, fat and protein in the fillet) between the two velocity groups were insignificant or of minor biological relevance. Muscle composition in smaller fish is, in several studies, reported to be affected by swimming exercise. Solstorm et al. (2015), found that juvenile salmon (98 g) at fast and moderate water velocity (1.5 and 0.8 BL/s) had lower fat content in the muscle compared with fish at slow velocity. Christiansen et al. (1989) observed that exercise (2.3–1.1 BL/s) was linked to decreased fat content and increased protein content in arctic char fry (1 g). In a study on larger Atlantic salmon post-smolt (1168 g) no differences in body composition of water, protein and fat were found with exercise (0.3–1.06 BL/s) (Grisdale-Helland et al., 2013). As a general observation, experiments with small fish use higher water velocity relative to body size compared to experiments with large fish, and this could be part of the explanation to different results. Studies for longer time periods with more sequential sampling would most likely provide more precise data on the relationship between water velocity, muscle cell recruitment, growth and fillet composition.

In trial 1, the MODERATE group had lower mean cathepsin activity than the LOW group. Significant differences was measured for cathepsin B and H, but not for cathepsin B + L. But with only two cages in each group, the cage effect reduced the strength of the study, and we evaluate the overall cathepsin activity (B, B + L, H) in the muscle tissue as reduced in the MODERATE group. Cathepsin is a large family of proteases that participate in protein degradation in lysosomes and endosomes, as well as in cytosol and the nucleus. Stress could be the cause of increased proteolytic activity, possibly mediated by elevated plasma cortisol (Mommsen et al., 1999). Environmental stress involving high fish density (≥125 kg/m^3) or low water flow (≤0.3 L/kg/min) increases cathepsin activity in salmon (Bahuaud et al., 2010; Sveen et al., 2016). The levels of cathepsin enzymes in the skin and muscle are therefore suggested as a possible stress indicator. If up-regulation of cathepsins indicates an adaptation to environmental stress, the results of this study indicate reduced stress in the MODERATE group.

4.3. Water velocity, swimming behaviour and fish welfare

The MODERATE groups were established with water velocity comparable with water velocity measured in commercial CCS (2780–6000 m^3 volumes, 20–25 cm/s, data not shown). The LOW groups were designed as a reference with approximately one-third of the velocity in the MODERATE groups. In all observations of the MODERATE groups and in a majority of observations of LOW groups, swimming activity was organised as circular schooling with a slow forward advancement. The true swimming speed in most observations for both groups was therefore slightly faster than the measured water velocity, with the exception of occasional bursts of activity connected to eating or rolling/jumping at the surface. The true swimming velocity was difficult to evaluate when the swimming activity in the LOW group broke down to more individual movement patterns. One possible bias is that the fish in the circular research cages could have been exposed to different water velocities. We observed that swimming speed was principally determined by water velocity, and the fish generally avoided the extreme velocities close to inlet or current boosters and close to the cage wall or the centre.

Swimming performance of salmonids is described in many studies. The salmon in this study were exposed to water velocities between 0.1 and 0.67 BL/s, and with an actual swimming speed somewhat faster than the water velocity. Critical swimming speed (U_{crit}) is defined as the maximum swimming speed before the fish reaches exhaustion (Brett, 1964; Tudorache et al., 2007). The U_{crit} of Atlantic salmon (408–491 g, 34.0–36.9 cm) across temperatures from 3 °C to 23 °C was determined by Hvas et al. (2017), with highest U_{crit} at 18 °C (93.1 ± 1.2 cm/s, 2.7 BL/s) and U_{crit} at 8 and 13 °C (the temperatures closest to our trials) of 2.3 and 2.6 BL/s respectively. Optimal swimming speed or U_{opt} is defined as the speed at which the cost of transport (COT) is lowest (Tucker, 1970; Beamish, 1978; Tudorache et al., 2007). A raceway study with post-smolt Atlantic salmon (98.6 g, 22.3 cm, 10 °C) at 0.2, 0.8 and 1.5 BL/s showed reduced performance and welfare at the highest swimming speed and best welfare at 0.8 BL/s (Solstorm et al., 2015). According to other authors the U_{opt} for salmonids is close to 1.0 BL/s (Thorarensen and Farrell, 2011; Drenner et al., 2012). In a study of

brook char Tudorache et al. (2007) defined a new measure for swimming performance, the preferred swimming speed or U_{pref}. For brook char (26.2 ± 0.6 cm, 12.2 ± 0.9 °C) the U_{opt} = 1.02 ± 0.47 BL/s and U_{pref} = 0.78–0.95 ± 0.03 BL/s were closely related, but data also showed that, during much of their time spent in a tilted raceway, the char preferred to swim at speeds ≤0.76 BL/s, and the authors suggest that a study of preferred swimming speed (U_{pref}) could be a way to determine welfare-friendly swimming speeds in aquaculture systems. A larger difference in water velocity between the groups in our trial could probably have increased the differences of some of the outcome variables in this study. Regulation of water flow and water velocity in such circular CCS systems is more complicated than in a raceway. In the LOW group, water velocity sometimes dropped towards levels where the schooling behaviour started to disintegrate. In the MODERATE group, the actual swimming speed was below the reported U_{pref} and U_{opt}, and could have been increased. It was technically difficult to increase the water velocity in this group, and more effort was invested in stabilising the velocities between the cages in each group. When comparing the environment inside netpen cages with CCS, there are several differences. In open sea cages the fish have to adapt to several important environmental factors such as light, oxygen levels and access to feed in a situation with temperature differences in the vertical water column and regular fluctuations of water velocity (Oppedal et al., 2011, Johansson et al., 2014). This forces the fish to adopt multiple behavioural trade-offs. In CCS the temperature gradient inside the cage is generally negligible (Nilsen et al., 2017a), and oxygen levels are controlled automatically through the built-in oxygenation systems. With stabilised temperatures and oxygen levels within the cage, water velocity could be adjusted to fish size, fish health and seasonal fluctuation of water temperatures. Spatial differences in water velocities inside the CCS could also be possible to utilise in order to provide an environment suitable for different individual behavioural needs or coping styles. In commercial scale CCS it will be necessary to determine the variation of water velocities and swimming speeds throughout the whole cage volume, how fish respond to this variation and how this again affects welfare, growth, muscle development and chemical composition.

In both trials it was necessary to transfer fish from the large cages to the research cages, but probably with a negative effect on the growth rates in both groups. The change of environment and more restricted volume could have a negative impact on behaviour and feed intake in both groups. Another factor contributing to the reduced growth in the LOW groups could be increased stress (Solstorm et al., 2015), possibly mediated by an increase in negative social interaction between the fish when water velocities are too low to support a stable, circular schooling behaviour. There were no differences in total mortality or mortality causes between the LOW and MODERATE groups. To transfer fish to the research cages, it was necessary to use netting and handling with the accompanying stress and risk of injuries such as loss of scales, even with precautions to minimise the negative impact of the research procedures. This could also have been the main cause of ulcer-related mortality in both trials. A trial with salmon of harvest size (4.8 kg, 67.3 cm) showed that exercise (35 to 70 cm/s) for periods between 1.5 and 12 h accelerated the recovery after crowding stress, compared to fish exposed to 0 cm/s (Veiseth et al., 2006). Applying higher water velocity to our closed cages during the acclimatisation period could have reduced the immediate stressful effect of handling and sampling.

The water quality throughout the study was good, and should not represent any risk of reduced growth performance or fish welfare. Long-term exposure to levels of CO_2 above 10–15 mg/L has been shown to cause reduced growth rate, increased feed conversion ratio (FCR) and nephrocalcinosis (Thorarensen and Farrell, 2011, Fivelstad et al., 2018). Recommended maximum concentration of CO_2 is 10 to 15 mg/L (Thorarensen and Farrell, 2011; Fivelstad et al., 2003; Fivelstad et al., 2018), for NH_3 0.012 to 0.025 mg/L (Fivelstad et al., 1995; Knoph and Thorud, 1995). For suspended solids, suggested maximum levels for long-term exposure are from 15 mg/L (Chen et al., 1993) to 80–100 mg/L (Wedemeyer, 1996). The trials were also designed to balance biomass, feed and water flow without exceeding recommended maximum levels of density, SWC and feed load.

5. Conclusion

Increase of water velocity from 0.1–0.2 BL/s (LOW) to 0.3–0.6 BL/s (MODERATE) enhances growth rates and muscle development in Atlantic salmon (300–3000 g, 7–11 °C). The main effects in two trials of 168 (trial 1) and 46 days' (trial 2) duration are increased body weight and condition factor (both trials), increased relative heart and liver size (trial 1), increased fillet yield (trial 1) and reduced levels of cathepsin activity in muscle tissue (trial 1). MODERATE water velocity had little impact on the chemical composition (protein, fat, water) of fillets (both trials) and the size distribution of muscle cells (trial 2). This study shows that a MODERATE water velocity is favourable for growth rates for Atlantic salmon during the entire on-growing period in CCS (300–3000 g). Indications of an effect on a broader range of metabolic variables and welfare indicators were also documented. These results should be tested with studies on a commercial scale CCS for longer periods (≥120 days), with more detailed sequential sampling procedures. Individual tagging of fish and parallel studies of fish behaviour and preferences would also add valuable information when interpreting such growth studies.

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Errata

Errata in Paper II:

Page number

10	Changed from:	"…FCR of 1.06 (Table 7)." (on line 308)
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