



CtrlAQUA

Annual Report 2015
CtrlAQUA - Center for Closed-
containment Aquaculture



Photo: Terje Aamodt ©Norfima

Centre for Closed-containment Aquaculture

sfi = Centre for
Research-based
Innovation
The Research Council of Norway

Content

1. Summary.....	3
2. Vision/objectives	5
3. Research plan/strategy	7
4. Organization.....	9
4.1. Organizational structure, and cooperation between the center's partners.....	9
4.2. Partners	11
R&D Partners.....	12
User Partners.....	16
5. Scientific activities and results.....	22
Common projects.....	22
DATABASE - Environmental and biological requirements & surveillance database for closed systems.....	22
BARRIER - Osmoregulatory barrier function in post-smolts reared in closed-containment systems.....	23
SENSOR - Sensor protection and maintenance in closed systems	27
RISK - Review of the microparasites that could represent a future problem for production of salmonids in closed or semi-closed containment systems.....	31
HYDRO - Hydrodynamic challenges in huge tanks (1000+ m ³).....	35
User specific projects.....	39
PARTICLE - Particle tolerance in post-smolts reared in RAS.....	39
BIOMASS - Machine vision for biomass in closed systems.....	42
REMOVAL - Pre-project, new technologies for RAS sludge processing	46
MICROPARASITES - Characterization of microparasites in closed and semi-closed containment systems.....	48
INTAKE - Particle and pathogen removal from intake water in semi-closed systems.....	52
FLEXIBAG - Water quality in Flexibag semi-closed system for post-smolts.....	56
PRELINE - Documentation of post smolt welfare and performance in large scale Preline semi-containment system (CCS).....	60
ROBUST - Robustness evaluation parameters associated with biological requirements in closed systems.....	64
6. International cooperation.....	67
7. Recruitment.....	67
8. Communication and dissemination activities.....	68
Attachments to the report:	70
Attachment 1: Personnel	70
Attachment 2: Accounting	72
Attachment 3: Publications	73

1. Summary

In the report “Creating value based on productive seas in 2050», it is predicted that Norwegian aquaculture will generate a 5x increase in volume and 8x in value by the year 2050. However, there are challenges that can hinder the industry in achieving this goal, such as sea lice, diseases, escapes, and fish mortality during production. Technological and biological innovations in closed-containment aquaculture systems, where the salmon’s environment is separated from the surrounding ecosystem by a closed barrier may be important for further development of the Norwegian aquaculture industry. Closed systems can be land-based, where water is recycled, or floating closed systems in the sea where clean water is pumped up from the deep.

CtrlAQUA is a Center for Research-based Innovation (SFI), funded by the Research Council of Norway and the partners. The center’s main goal is to develop technological and

biological innovations to make closed-containment aquaculture systems a reliable and economically viable technology. The main focus is innovations for the most vulnerable periods in the salmon production cycle, such as the first salt-water phase, the so-called post-smolt stage. In this way, the salmon aquaculture industry can get a good alternative or supplement to the current production technology with open cages. The center will also contribute to improved production control, animal welfare and sustainability in closed systems.

This annual report covers activities during the first nine months of the center. In 2015, 14 projects were initiated in CtrlAQUA, whereas for 2016 a portfolio of 18 projects was approved by the Board. The projects cover all the objectives of the center and will collectively contribute to the most important innovation, making production of post-smolts in closed systems reliable and efficient. A high



Figure 1.1. CtrlAQUA partners gathered at the kick-off meeting, 27th – 28th May 2015 at Nofima, Sunndalsøra (Photo: Terje Aamodt ©Nofima)



(Photo: Terje Aamodt ©Nofima)

degree of interdisciplinary activities characterize the center. The projects range from hydrodynamic models of land-based culture tanks in HYDRO, to large-scale experiments with floating closed-containment systems in sea in PRELINE, to how fish barrier functions are affected in land-based facilities in the BARRIER project. In this report, we present summaries of the research in each of the projects in 2015.

The experiments during the first year have provided additional data indicating that closed-containment aquaculture systems lead to reduced sea lice counts, increased survival and comparable growth rates to traditional cage-based systems (projects PRELINE, FLEXIBAG). Furthermore, the research in HYDRO has resulted in new knowledge on how water flow rates and velocities in large culture tanks currently are dimensioned, and identified limitations that need to be addressed to ensure good mixing and water quality in the huge tanks of the future. Studies on physiology and welfare of Atlantic salmon post-smolts in closed systems, such as in the ROBUST project, have resulted in new indicators for

robustness and health that will be developed in the coming years for operational use. In BARRIER, experiments in recirculating and flow-through systems have shown that low chronic stress followed by acute stress can influence skin permeability, which may render the fish more sensitive to later challenges and toward pathogens. Such findings are essential in developing production protocols and welfare indicators for closed-containment systems. A substantial review on health and disease in closed-containment systems was undertaken in RISK, which will prove valuable for identifying the particular pathogens that should be focused on in the coming years in the center. Furthermore, in total four PhD students, one post-doc, and five MSc students are already associated with CtrlAQUA, a good contribution to the goals for recruitment in the center. In conclusion, through their work in the projects all the partners have contributed significantly to the good results this first year, and thereby secured the foundation for successful years ahead in the center.

Bendik Fyhn Terjesen,
Centre Director, CtrlAQUA SFI

2. Vision/objectives

In the report "Value created from productive oceans in 2050", aquaculture is expected to increase 5x in volume and 8x in value. The Norwegian authorities and salmon industry work towards this vision. But there are challenges that may hinder achievement of this goal, such as sea lice, diseases, escapes, and the loss of fish through production. Innovations in closed-containment aquaculture systems, where the salmon is separated from the outside environment by a closed barrier, can be important for further development of aquaculture.

CtrlAQUA is a center for research-based innovation (SFI) that will work on such closed-containment systems. The main goal is to develop technological and biological innovations that will make closed systems a reliable and economically viable technology. Closed systems can be land-based where

water is recycled, or sea-based, in which large floating tanks receive clean water from depth. In CtrlAQUA the research will deal with both approaches.

Focus will be on the most sensitive phases for the salmon in the production cycle, such as the first seawater phase, the so-called post-smolt stage (Figure 2.2). The main innovation will be reliable and efficient production of post-smolts in closed systems on land or at sea. Thus, the industry can get a good realistic alternative or supplement to the current production technology with open cages. The center will also contribute to better production control, fish welfare and sustainability in closed-containment farms. This will happen through development of new and reliable sensors, minimizing environmental impact through recycling of nutrients and reduce the risk of escape, and diseases transmission to



Figure 2.1. Minister of Fisheries and Coastal Affairs, Elisabeth Aspaker, visited Nofima Sunndalsøra during the CtrlAQUA kick-off in May 2015 (Photo: Terje Aamodt ©Nofima)

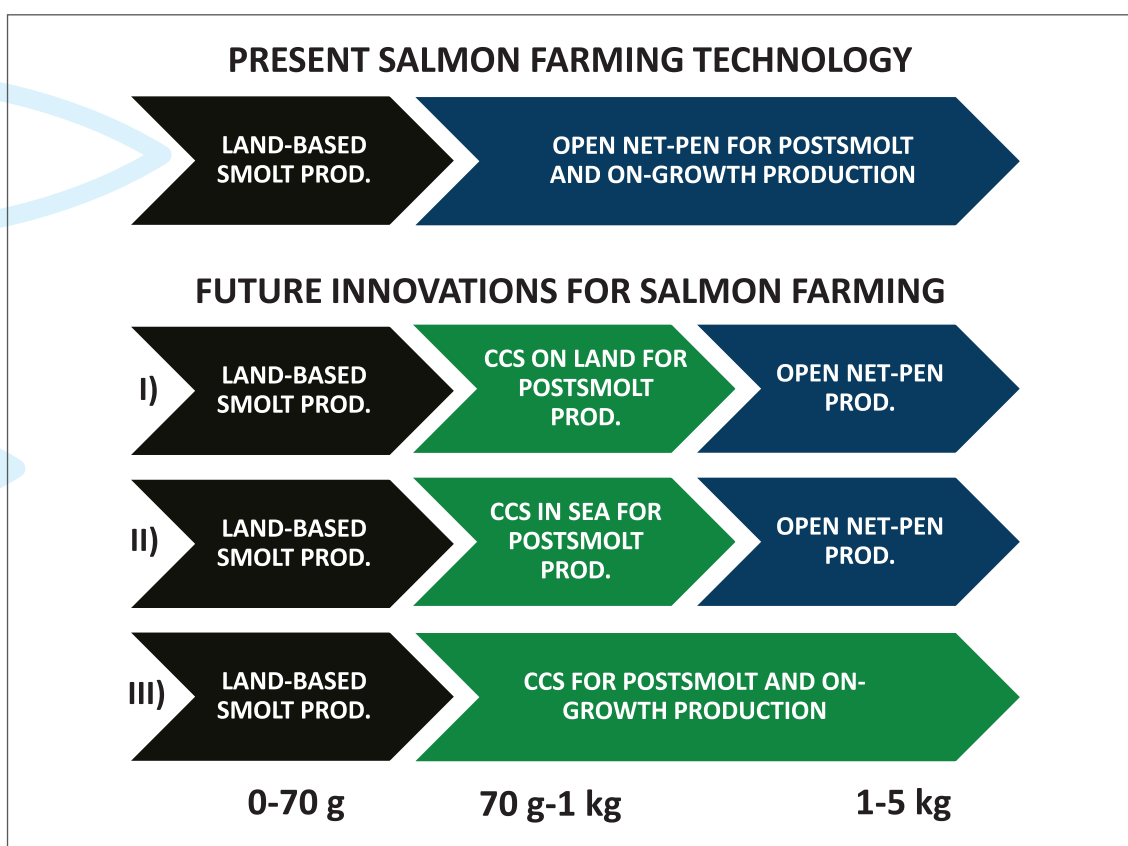


Figure 2.2. Present salmon farming technology, and future innovative strategies (I-III). CCS: Closed-containment aquaculture systems.

wild stocks. These innovations will be of value to the Norwegian society, since closed systems for strategic phases in salmon farming can help to make the vision of an eight-fold growth in value creation from aquaculture possible, and lead to increased number of jobs and production of healthy seafood.

Therefore, the main goal of CtrlAQUA SFI is to:

Develop technological and biological innovations to make closed-containment aquaculture systems (CCS) a reliable and economically viable technology, for use in strategic parts of the Atlantic salmon production cycle, thus contributing significantly to solving the challenges limiting the envisioned growth in aquaculture

The secondary goals of CtrlAQUA are to:

1. Innovate closed-containment aquaculture system technology in sea and on land
2. Develop and implement water treatment processes and sensors, to increase control in CCS production
3. Develop knowledge and innovations on environmental and biological requirements of salmon for engineering and improve management of CCS
4. Develop new innovative tools to increase pathogen control, minimize disease risk and strengthen health of salmon in CCS
5. Promote knowledge-transfer to user partners, to ensure implementation in products and markets
6. Disseminate knowledge to users, scientists and the public and educate 15 PhDs in relevant fields

3. Research plan/strategy

The Center for Research-Based Innovation in Closed Containment Aquaculture, CtrlAQUA, commenced operations in April 2015.

The Research Council of Norway's objectives in running the SFI-program are four-fold: 1) to stimulate innovation activities in strong industries in Norway, 2) to promote collaboration between innovative industries and excellent research institutions, 3) to develop industry-relevant research institutions that are leading in their field, and 4) to educate new scientists and foster knowledge- and technology transfer.

These goals, in addition to the specific goals of the center, forms the basis for the work in CtrlAQUA. Through close collaboration between user partners and the R&D institutions, the center focus on new closed-containment system innovations, such as new RAS process units and methods for improved fish welfare and health, shown in Figure 3.1. In the annual plan for 2015, the first operative year of the center, the overall focus was to start several

strong research lines that will make it possible to reach these goals during the center lifetime.

The work on the research plan was led by the leader group of CtrlAQUA, who used several sources of information to develop the plan, including: the SFI Center Description which was part of the proposal in 2014, the Letters of Intent by the user partners, meetings with the user partners in 2014 and 2015, and inputs received from the partners during the kick-off meeting held May 27-28 at Nofima, Sundalsøra. A Scientific Advisory Board (SAB) was appointed for CtrlAQUA, consisting of researchers and stakeholders with competencies in the fields of research in the center. An important task of the SAB in the years ahead will be to advice during development of the annual plans.

In total 14 projects was approved by the Board for 2015. These projects were divided into common projects and user-specific projects, according to the guidelines agreed on

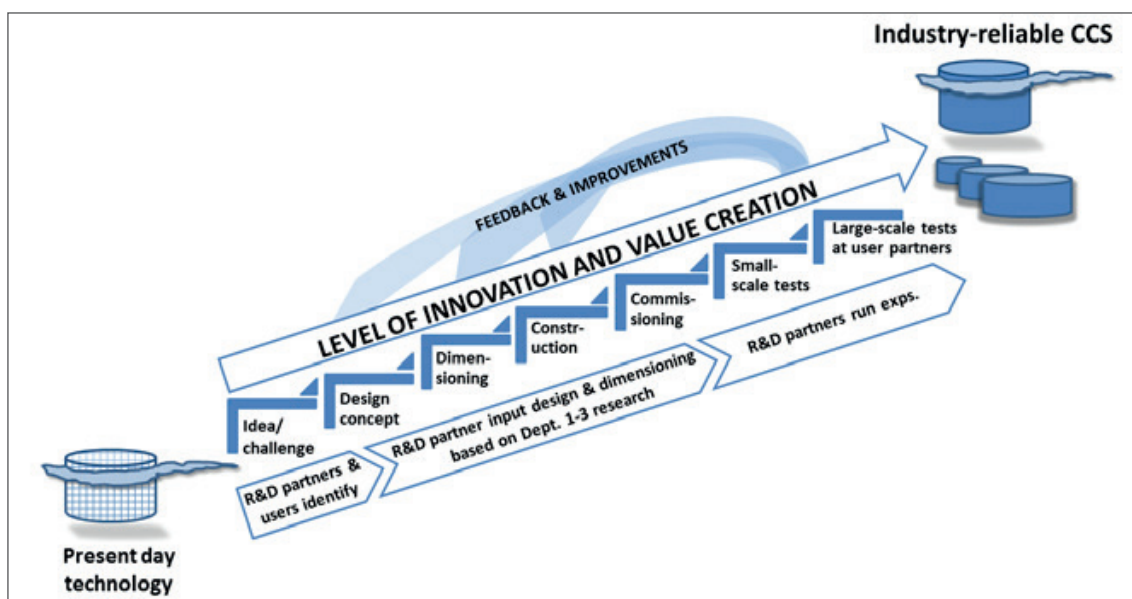


Figure 3.1. Innovation process in CtrlAQUA, from present day cage technology, to establishment of industry-reliable closed containment systems, either in-sea closed floating systems or land-based RAS. Exps': experiments. RAS, Recirculating Aquaculture Systems.

in the CtrlAQUA Center Description and Consortium Agreement. Both types of projects will contribute towards the main goal of the center stated above. Common projects are defined as activities that benefit all partners in the center, such as environmental requirements of salmonids in closed-systems or hydrodynamic modelling. User-specific projects are defined as activities which are particularly important for one user partner, or a group of user partners.

The roadmap for the innovations and research lines in CtrlAQUA are shown in Figure 3.2. Here, several innovation deliverables are included, that collectively leads towards the main goal of the center (see Figure 3.2, i1.1-i11.4). These deliverables are further linked to the Departments and their specific research lines in the center (rows in Figure 3.2). For each of the 14 projects in the annual plan for 2015, a box is included in Figure 3.2 to identify which innovation deliverable that the particular project contributed to.

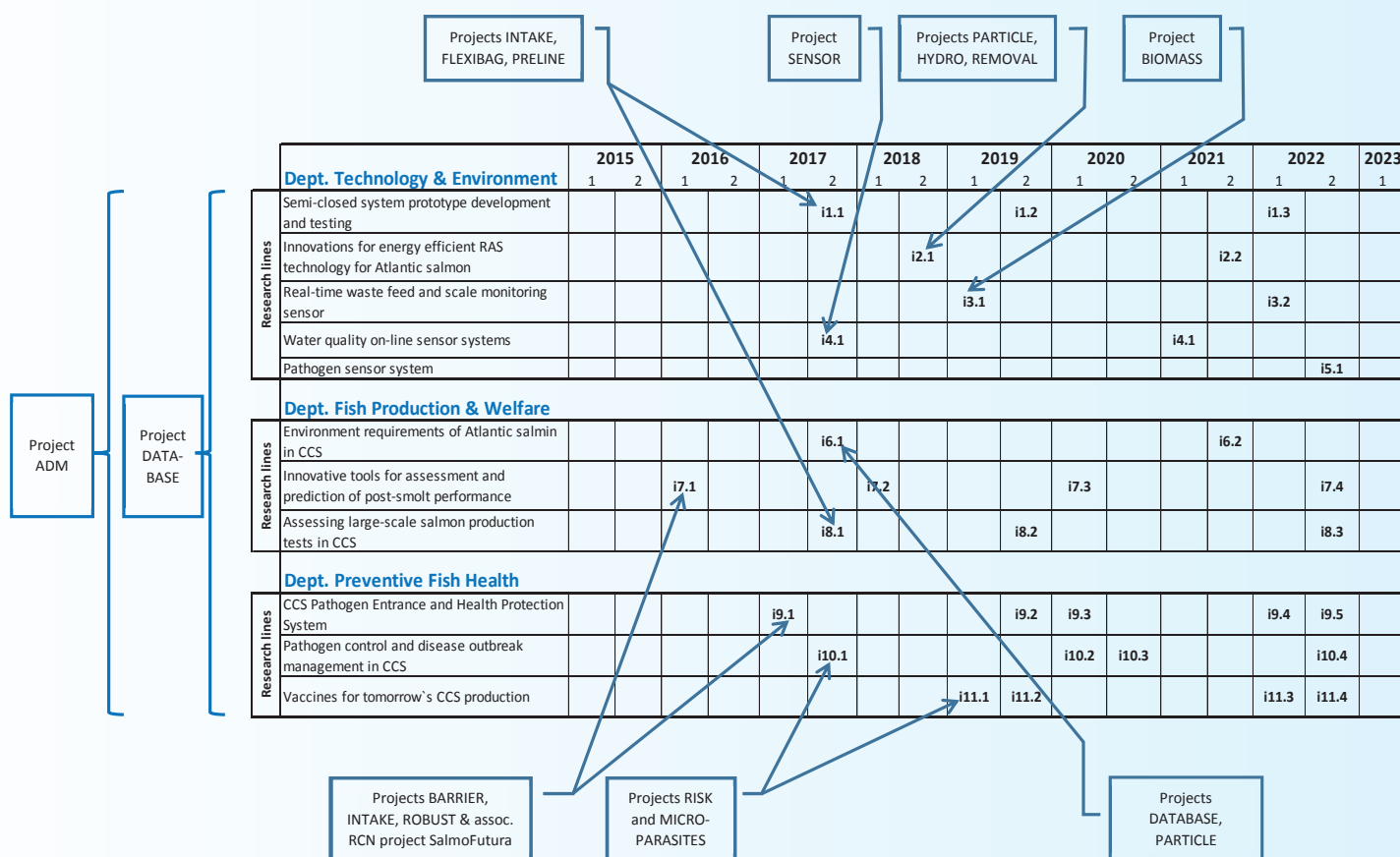


Figure 3.2. Relationship between the overall CtrlAQUA innovation deliverables and research lines, and the 2015 CtrlAQUA projects.

4. Organization

4.1. Organizational structure, and cooperation between the center's partners

CtrlAQUA is organized (Fig. 4.2) with a Board that oversees that obligations are fulfilled, and decides on financial, partnership, and IPR issues, as well as ratifying annual research plans made by the leader group. In 2015, the Board consisted of the following elected members (Fig 4.1):

- Frode Mathisen, Grieg SeaFood, chairperson of the CtrlAQUA Board
- Harald Sveier, Lerøy SeaFood Group, Board Member
- Knut Måløy, Storvik Aqua, Board Member
- Siri Vike, Pharmaq Analytiq, Board Member
- Tor Solberg, UNI Research, Board Member
- Mari Moren, Nofima, Board Member and representing the Host institution

In addition, Kjersti Turid Fjalestad, the contact person for CtrlAQUA at the Research Council of Norway, has been observer at the Board meetings.

The center scientific work is organized through close collaboration between three

departments: Dept. Technology & Environment, Dept. Fish Production & Welfare, and Dept. Preventive Fish Health, whereas student recruitment is managed in Dept. Training & Recruitment.

The leader group manages and leads CtrlAQUA, such as ensuring planning and running of experiments, recruitment of qualified personnel, and providing a good working environment.

In CtrlAQUA there has been a strong focus on collaboration and knowledge transfer between the partners during 2015. This collaboration has been done within the projects, and occurred between R&D partner scientists, scientists and user partners, and between user partners, as is described in the project result section below. The extensive collaborations through 2015 was facilitated by participation from all institutions in project workshops, as well as joint experiments, sampling and analytical work. Further, research tasks was undertaken at user partner large-scale facilities, such as Dept. Fish Prod & Welfare and Dept. Tech & Env on semi-closed system large scale farming sites (e.g. PRELINE), and RAS culture tanks (e.g. HYDRO), respectively.

Frequent meetings were organized at Board level (each six months), Center level (kick-off in May 2015), Dept. level (each month), leader group (each second week), and at project level as required. In addition, we set up the CtrlAQUA intranet as a key communication channel. The intranet has a news feed where center-participants have posted e.g. news, links to documents, research plans, results and pictures. In addition to a formal news channel, the center intranet has also been used as a social media, thus contributing to build the CtrlAQUA team spirit.



Figure 4.1. The CtrlAQUA board. Back left: Harald Sveier, Knut Måløy, Tor Solberg. Front left: Siri Vike, Frode Mathisen, Mari Moen (Photo: Terje Aamodt ©Nofima)

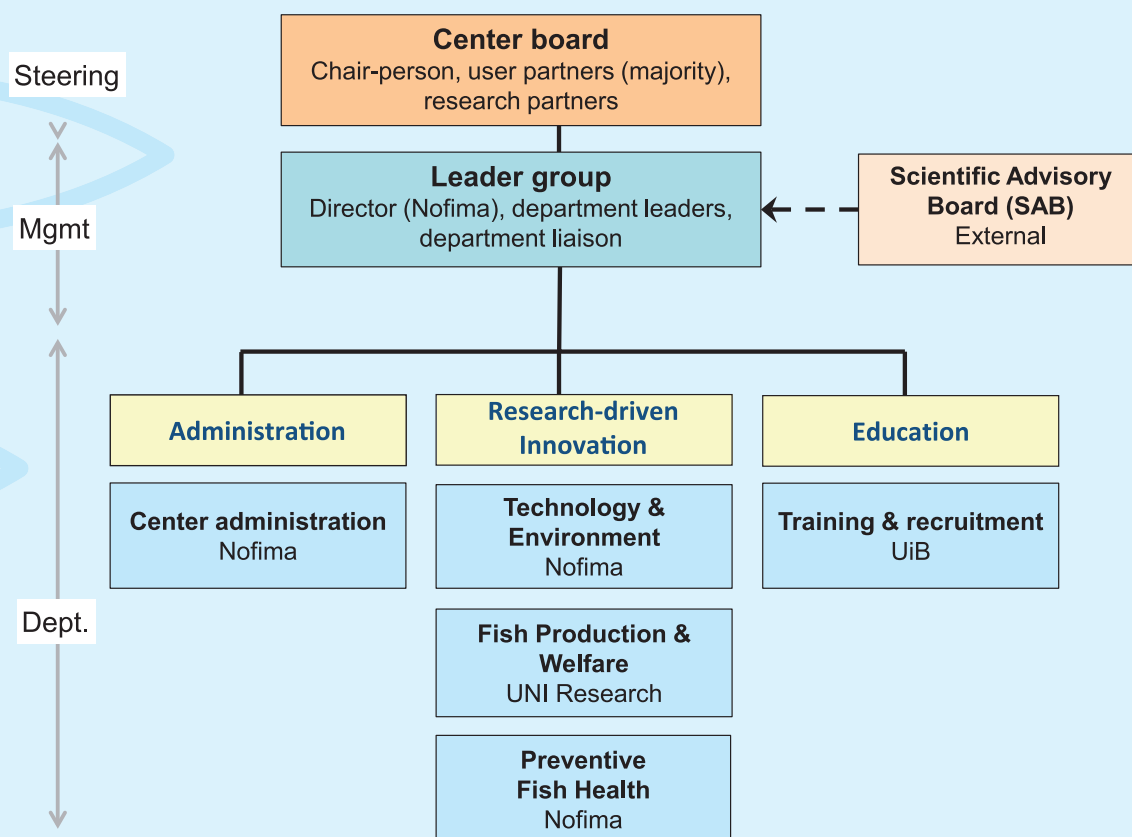


Figure 4.2. Organizational structure of CtrlAQUA. Although, for instance Dept. Technology & Environment is led by Nofima, all partners contribute to the research in all departments.



Figure 4.3. Technician Britt Kristin Megård Reiten from Nofima measure water quality in a closed-containment system.

4.2. Partners

Per April 1, 2016, CtrlAQUA has 20 partners, of which six are R&D partners and 14 are user partners (Table 4.1).

Table 4.1. List of partners in CtrlAQUA per 1st April 2016

Partner name	Remarks
R&D Partners	
1. Nofima (host institution)	
2. UNI Research	
3. University of Bergen (UiB)	
4. Norwegian University of Science and Technology (NTNU)	
5. The Conservation Fund Freshwater Institute (USA)	
6. University of Gothenburg (Sweden)	
Farming User Partners	
1. Bremnes Seashore AS	
2. Cermaq Norway AS	
3. Firda sjøfarmers AS	Left the center 22. June 2015
4. Grieg Seafood ASA	
5. Lerøy Seafood Group ASA	
6. Marine Harvest ASA	
7. Smøla klekkeri og settefiskanlegg AS	
Technology User Partners	
1. Aquafarm Equipment AS	
2. Krüger Kaldnes AS	
3. Oslofjord Ressurspark AS	
4. Storvik Aqua AS	
5. FishGlobe AS	Member of the center 17th November 2015
6. Botngaard AS	Member of the center in 2016
Biotechnology User Partners	
1. Pharmaq Analytiq AS	
2. Pharmaq AS	

There have been a few changes in the list of user partners:

- Firda Sjøfarmers AS left the consortium after approval at the board meeting 22nd June 2015.
- FishGlobe AS was approved as a new member of the consortium at the board meeting 17th November 2015.
- Botngaard AS was approved as a member of the consortium in 2016, and their membership will therefore first be described in the 2016 annual report.

R&D Partners

Nofima AS (host institution)

Nofima is the host institution of CtrlAQUA, the contract partner for RCN, and bear the practical, scientific and financial responsibility for all activities of the Center. Nofima is one of the largest institutes for applied research within the fields of fisheries, aquaculture and food research in Europe. Nofima supply internationally renowned research and solutions that provide competitive advantages along the complete chain of value. The head office is located in Tromsø, with research activities in Alta, Bergen, Stavanger, Sunndalsøra, Tromsø and Ås. Bendik Fyhn Terjesen (Nofima Sunndalsøra) is centre director and leads CtrlAQUA, as well as leader of Dept. Tech & Env. Nofima is leading or participating in all

Depts, and contributes with significant in-kind funding. Sven Martin Jørgensen at Nofima Ås is leading Dept. Prev Fish Health, as well as being part of the Leader Group. The Leader Group also contain Nofima employees Astrid Buran Holan (meeting organizer), and Åsa Maria Espmark (responsible for reporting, internally and to RCN). Nofima is providing modern closed-containment system research facilities, state-of-the-art biotechnology labs, and meeting and guest accommodation at all main locations of the Center. Nofima provides administration personnel for CtrlAQUA with competences in coordination, IPR, and contract law, to assist all partners. The Nofima personnel is further supported by Nofima accounting, IT, and management.



Figure 4.4. Nofima is the host institution of CtrlAQUA. Much experimental work will be done at the research station in Sunndalsøra (Photo: Kjell Merok ©Nofima)



Some Nofima researchers and technical personnel attached to the center. From left: Roger Selset, Astrid Buran Holan, Bendik Fyhn Terjesen, Magne Nordvik, Jelena Kolarevic, Frode Nerland, John Andrè Nordmann, Yuriy Marchenko. Not present: Britt Kristin Megård Reiten, Åsa Maria Espmark, Turid Synnøve Aas, Trine Ytrestøyl, Vasco Mota and Jagan Gorle (Photo: Terje Aamodt ©Nofima)

Uni Research

Uni Research in Bergen is a broadly based, multidisciplinary research institute with 450 employees from 40 different nations. Uni Research carries out research and development in the fields of biotechnology, health, environment, climate, energy and social sciences. The Institute comprises six thematic departments and one subsidiary, Uni Research Polytec. The Integrative Fish Biology group in the Uni Research Environment department is a leading R&D partner within CtrlAQUA, with multidisciplinary researchers and technicians (Figure 4.5). Uni research is involved in all CtrlAQUA Departments where Prof. Lars Ebbesson leads the Dept. Fish Prod & Welfare, member of the

Leader Group, and leads the development of robustness indicators. Dr. Sigurd Handeland is Liaison between Departments, R&D and User Partners, member of the Leader group and leads the development of the semi-CCS Preline and Neptune. Uni Research leads all experiments that occur at ILAB. Finally, UNI contributes with significant in-kind funding via scientific, technical and management man-hours.

University of Bergen (UiB)

University of Bergen, Department of Biology (BIO) is among the largest departments at the University of Bergen and has more than



Figure 4.5. The Integrative Fish Biology group at Uni Research in Bergen. From left to right: Prof. Lars Ebbesson (Leads Dept. Fish Production & Welfare), Dr. Tom Ole Nilsen (Senior Researcher and Task leader), Dr. Elsa Denker (Postdoc), Dr. Sigurd Handeland (Researcher I, Dept. Liaison), Valentina Tronci (Senior Dept. Engineer), Dr. Marco Vindas (Researcher), Cindy Pedrosa (Dept. Engineer): Not in picture; Dr. Pablo Balseiro (Researcher) and Adjunct Senior Researchers Profs. Simon Mackenzie, Victoria Braithwaite and Prof. Hans Hofmann.

50 permanent scientific positions, more than 50 technical or administrative positions, and more than 100 temporary scientific positions, including PhDs and post docs. BIO undertakes research across a broad range of disciplines: developmental biology, microbiology, evolutionary biology, ecology and biodiversity. BIO is also strongly involved in themes such as climate, aquaculture, fish health, nutrition, fisheries, and petroleum. BIO has the responsibility for the education and training of young scientists in CtrlAQUA, led by Prof. Sigurd Stefansson and Prof. Are Nylund. Prof. Sigurd Stefansson is in addition member of the Leader Group. BIO, by Prof. Are Nylund, is leading Task 2 of Dept. Prev Fish Health,

on disease outbreak management in closed systems. The PhD candidates will be enrolled at UiB, and in some cases also work at UiB, but also in several cases being affiliated with other R&D partners, e.g. Nofima. BIO also provide in-kind through access to advanced laboratory facilities for state-of-the-art techniques, and personnel hours.

Norwegian University of Science and Technology (NTNU)

Department of Chemistry at Norwegian University of Science and Technology (NTNU), based in Trondheim, offers both theoretical and practical areas of study, and a wide

variety of research areas including theoretical chemistry, environmental and analytical chemistry, organic chemistry and structural chemistry. NTNU is predominately connected to Dept. Tech & Env., and is contributing with water quality sensor development, sensor testing, sensor implementation, and establishment of sensor maintenance routines, led by Prof. Øyvind Mikkelsen. Several PhD students will be enrolled at NTNU for CtrlAQUA research, and supervised by NTNU, in close collaboration with user partners. NTNU also provides in-kind, distributed between use of advanced laboratory infrastructure, hours for scientific staff and engineers as well as co-financing PhD's.

The Conservation Fund Freshwater Institute UGOT (USA)

The Conservation Fund creates conservation solutions that make environmental and economic sense. The Fund's Freshwater Institute (FI) specializes in the production technology and design of aquaculture systems; and in solutions to the water quality constraints and impacts presented by farms and communities to meet the challenge of doubling the global food supply by 2050. FI is participating in Dept. Tech & Env, and Dept. Prev. Fish Health

by providing extensive advisory and technology support to user partners in Norway, and by providing research from its facilities in Shepherdstown, WV. The work is led by Steven Summerfelt, Brian Vinci, and Chris Good. FI commits resources through in-kind research hours, matching funding from U.S. foundations or corporations, and experimental facility fees.

University of Gothenburg UGOT (Sweden)

University of Gothenburg (UGOT), The Department of Biological and Environmental Sciences (BioEnv) is located in the Botanical Garden and on Medicinareberget in Gothenburg and Kristineberg (Fiskebäckskil) at the Sven Lovén center for Marine Sciences. The activity is led by acting Operational Officer Lars Förlin. The Primary Barrier group, contributes with the combined expertise of their whole research group, led by Prof. Kristina Sundell. In CtrlAQUA, UGOT is focusing on Depts. Fish Prod & Welfare, and Prev Fish Health. The contribution consists of researchers involved in the activities of the Center, and with in-kind through salaries, and infrastructure access to the fish physiology lab with equipment for running several state-of-the-art laboratory techniques.



Figure 4.6. Atlantic salmon grown to market size in land-based closed-containment at the Freshwater Institute (Photo: Kata Sharrer).

User Partners

Bremnes Seashore AS

Bremnes Seashore AS is one of Norway's leading suppliers of farmed salmon. Research and development have given them their own, patented production processes, and they established SALMA as Norway's first brand for fresh fish. Bremnes Seashore currently handles the full production chain for salmon, and are one of the largest privately owned salmon farming companies in Norway. The company has farming facilities in Hardanger, Sunnhordland and Rogaland, which are spread across

23 locations in 9 different municipalities. In CtrlAQ-UA, Bremnes Seashore is represented by Farming Manager Geir Magne Knutsen, and will contribute with their facilities at Tronvåg with RAS-technology during testing.

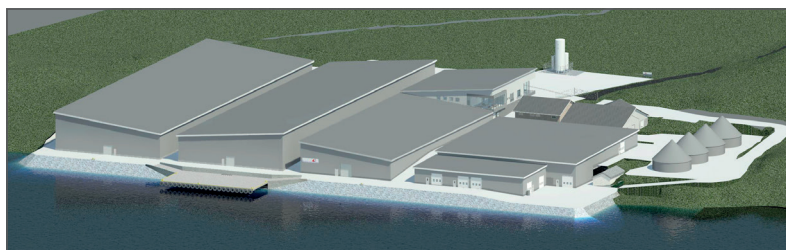


Figure 4.7. Bremnes Seashore AS RAS facility at Tronvåg (Illustration: Bremnes Seashore AS)

Cermaq Norway AS

Cermaq Norway produces Atlantic salmon with operations in Nordland (22 licenses and two processing plants) and in Finnmark (27 licenses and one processing plant). The three freshwater sites are all located in Nordland. Cermaq vision is to be a leading aquaculture company in Norway, through sustainable salmon farming. Fundamental to this work is Cermaq Norway's preventative health strategy for fish. This means using the knowledge of the salmon's biology, physiology and environment, to achieve the best fit between production, fish welfare and growth. In CtrlAQ-UA, Cermaq Norway is represented by R&D Manager Olai Einen. He has extensive background in research, R&D management, fish nutrition and product quality. Cermaq will also contribute with their fish health group, and closed system testing facilities.



Fig 4.8. Cermaq farming in Slettnes in Finnmark (Photo: Cermaq)

FishGlobe AS

FishGLOBE is a company that has developed, built and is testing a new solution for closed aquaculture. The solution is patent pending. The company was established in 2013, but the development of closed aquaculture technology has roots back to the late 80's. Then a closed

solution was developed in concrete together with AS Betong. The company is located in Forsand, Norway. The vision of FishGlobe is to develop new cost-effective solutions that makes it possible for the aquaculture industry to expand. The business concept is to offer a solution to the salmon farmers that make farming more profitable, more sustainable and with higher fish welfare. FishGlobe entered CtrlAQUA in November 2015, and is represented by Director Arne Berge.



Fig 4.9. FishGlobe 's V5 Postsmolt (Illustration: FishGlobe)

Grieg Seafood ASA

Grieg Seafood ASA is one of the world's leading fish farming companies, specializing in Atlantic salmon. They have an annual production capacity of more than 90.000 tons gutted weight. The Group is today present in Norway, British Columbia (Canada) and in Shetland (UK), employing approximately 700 people. Grieg Seafood ASA was listed at the Oslo Stock Exchange (OSEBX) in June 2007. The headquarters are located in Bergen, Norway. The business development of Grieg Seafood ASA focuses on profitable growth, sustainable use of resources and being the preferred supplier to selected customers. Grieg Seafood is represented in CtrlAQUA by Director Biological Performance and Planning Frode Mathisen, who is also the chairperson of the Board of CtrlAQUA. Grieg Seafood will contribute with their long experience in salmon aquaculture and RAS, as well as running large-scale trials.

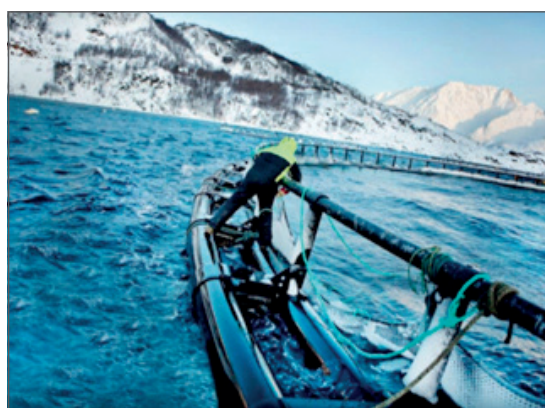


Figure 4.10. From Grieg Seafood i Finmark (Photo: Grieg Seafood)

Lerøy Seafood Group ASA

Lerøy Seafood Group is a leading exporter of seafood from Norway and is in business of meeting the demand for food and culinary experiences in Norway and internationally by supplying seafood products through selected distributors to producers, institutional households and consumers. The Group's core activities are distribution, sale and marketing of seafood, processing of seafood, production of salmon, trout and other species, as well as product development. The Group operates through subsidiaries in Norway, Sweden, France and Portugal and through a network of sales offices that ensure its presence in the most important markets. Lerøy Seafood Group's vision is to be the leading and most profitable global supplier of quality seafood. In CtrlAQUA, Lerøy



Figure 4.11. Lerøy Seafood Preline S-CCS (Illustration: Preline, Photo, Lerøy)

is represented by Technical Manager Harald Sveier, who has a long research background in fish physiology and nutrition. Sveier, who is also a member of the CtrlAQUA board, will head Lerøy's work in developing closed-containment systems, and the testing-site Samnanger.

Figure 4.11. Lerøy Seafood Preline S-CCS (Illustration: Preline, Photo, Lerøy)



Marine Harvest ASA

Since the precursors of Marine Harvest started up in 1965, they have gone from a small entrepreneurial company to the world's largest aquaculture company. With 3.8 million daily meals, Marine Harvest in Norway is the largest food producer (in proteins) through the entire value chain from feed production to brood, eggs, fish, processing and distribution to sales. Most of the salmon from operations in Norway is exported to Europe, USA and Asia. Marine Harvest develops future solutions for farming and is a key driver for innovation, both in Norway and internationally. Business in Norway include being the largest aquaculture company in Norway with over 1600 employees and with operations along the Norwegian coast from Flekkefjord in Agder to Kvænangen in Troms. The Norwegian production is divided into four geographic regions: North, Central, West and South. The company is part of the group Marine Harvest ASA, which operates in 24 countries and is listed on the Oslo Stock Exchange (OSE) and New York (NYSE). The global headquarters are located in Bergen. In CtrlAQUA Marine Harvest is represented by Group Manager Technology Ragnar Joensen, and Fish Health Specialist Harald Takle and Industry-PhD student Sara Calabrese. In addition to the closed-containment system site at Molnes, Marine Harvest RAS sites such as Steinsvik is also provided to CtrlAQUA.



Figure 4.12. Marine Harvest, Molnes (Hordaland county, Norway), and Steinsvik RAS (Møre and Romsdal county) (Photos: upper photo, Steve Summerfelt, bottom photo, Bendik Fyhn Terjesen)

Smøla klekkeri og settefiskanlegg AS

Smølen Handelskompani AS is a holding company placed in Smøla County, Norway. The company owns Smøla Klekkeri og Settefiskanlegg AS and Sagafisk AS that together have a production capacity of 5.5 million salmon smolt per year. Initially the company was started up in 1984, and in 1999 it was invested in eel farming. The farm also has a cod license, but today's activities are hatching until harvest of trout and salmon. Smøla Klekkeri og Settefiskanlegg is represented in CtrlAQUA by Managing Director Per Gunnar Kvenseth, and contributes with expertise on RAS and floating closed-containment systems in sea, and facilities and personnel for testing new closed-containment system concepts.



Figure 4.13. Smøla Klekkeri og Settefiskanlegg. Floating semi-closed containment system (Photo: Smøla Klekkeri og Settefiskanlegg)

Aquafarm Equipment AS

Aquafarm Equipment AS is the world's leading designer and producer of floating closed fish cage systems. The Neptun Cage has proven to give many advantages; no chemicals required for delousing, improved Feed Factor (FCR), reduced mortality, no escapes, controlled water flow and oxygen saturation, etc. The new generation of Neptun is now ready for sale. In CtrlAQUA, Aquafarm Equipment AS is represented by engineers CEO Atle Presthaug, and project manager Arne Henry Nilsen, and contribute with their expertise in engineering of floating closed-containment systems in sea.

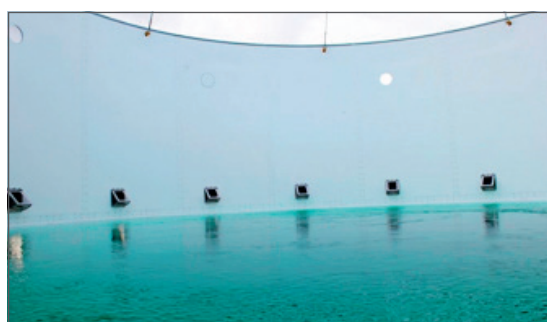


Fig 4.14. The Neptun closed cages from Aquafarm Equipment AS (Photo: Aquafarm Equipment)

Krüger Kaldnes AS

Krüger Kaldnes AS is an internationally oriented, Norwegian company that provides total solutions for wastewater treatment, water treatment, sludge treatment, rehabilitation and services to Municipalities and Industry in Norway. As part of Veolia Water, Krüger Kaldnes can offer a diverse range of world-leading technologies and products. The company is best known for Kaldnes®MBBR process, which is the market leader in the world and meets EU requirements for tertiary treatment. Known technologies in aquaculture is Kaldnes® Recirculating Aquaculture

System (RAS), which includes particle separation with Hydrotech drum filters and biological treatment with Kaldnes® MBBR. In CtrlAQUA, Krüger Kaldnes is represented by VP Aquaculture Marius Hægh, and R&D Manager Yngve Ulgenes, who has 23 years of research background in SINTEF on water treatment technologies. Kaldnes will contribute with own expertise, and prototype hardware.



Figure 4.15. Krüger Kaldnes AS Kaldnes® RAS (Photo: Krüger Kaldnes)

Oslofjord Ressurspark AS

Oslofjord Ressurspark (ORP) is a Norwegian commercial company delivering a point-of-care instrument and a disposable chip for automatic sample processing, sample refining and analysis of gene activity. ORP was established in 2013, and based its business on unique technology that is covered by own patents invented by the team of professor Frank Karlsen and licensed patented technology from PreTect AS. The strategy for ORP is to successfully sell and deliver products to customers in the international fish farming arena which will pave the way for other major markets in the oil, health, environment and agriculture fields. ORP is initially focusing on the supply of products on site for automatic and accurate detection of active fish genes and pathogenic micro-organisms in closed or open aquaculture facilities. In CtrlAQUA, ORP is represented by Business and Coordinator Manager Steve Hughes, and they will contribute with developing the pathogen sensor, together with CtrlAQUA partners (R&D, and user partners) for in-depth knowledge of relevant pathogens.

Storvik Aqua AS

Storvik Aqua is a Norwegian equipment supplier that has worked to help customer profitability to increase in correlation with fish welfare for over 30 years. The aim is to constantly develop, produce and deliver sustainable and eco-friendly solutions, designed to improve production for our customers. The products focus on oxygen (adding, logging and adjusting), logging of



Figure 4.16. AquaVision (camera based biomass estimator) is one of Storvik Aqua's products that will be used in CtrlAQUA

environmental data, biomass measuring, tools for closed cage treatment, and feeding equipment for land- and sea. In CtrlAQUA, Storvik Aqua is represented by chairman Knut Måløy (also member of the CtrlAQUA Board), and CEO Svein Arve Tronsgård, and will contribute to Dept. Tech & Env. with equipment prototypes, and expertise.

Pharmaq Analytiq AS

Pharmaq Analytiq is a Norwegian biotechnology company. Havbruksinstituttet AS was founded in year 2000 to provide services in the industry. The services were counseling, biological quality assurance and production optimization. In 2008 the company opened its state-of-the-art real time RT-PCR laboratory for the detection of pathogens. The laboratory was accredited by Norwegian Accreditation in January 2011. Havbruksinstituttet AS was in 2012 bought by PHARMAQ Holding AS, and changed its name that year to PHARMAQ Analytiq. Analysis and advice on smoltification and infection monitoring is today the main aims of the company. They are specialized in analytical services and consulting to improve fish health and welfare in aquaculture. In CtrlAQUA, PHARMAQ Analytiq is represented by General Manager Dr. Siri Vike, who is also a member of the CtrlAQUA Board, and R&D Manager Dr. Stian Nylund, who both have an extensive research background in fish health. PHARMAQ Analytiq will contribute in development of tools for assessment of salmon post-smolt robustness, and pathogen tests.

Pharmaq AS

PHARMAQ is the world's leading pharmaceutical company supplying the aquaculture industry and part of Zoetis, the world leader in animal health. The company provides environmentally sound, safe and efficacious health products to the global aquaculture industry through targeted research and the commitment of dedicated people. The vaccines are manufactured in a state-of-the-art production facility in Overhalla and Oslo, Norway. Administration and research and development activities are based in Oslo with subsidiaries in Norway, Chile, United Kingdom, Vietnam, Turkey, Spain, Panama and Hong Kong. PHARMAQ has approximately 200 employees. The company's products are marketed in Europe, North and South America, and Asia. In CtrlAQUA, PHARMAQ is represented by Technical Manager Nils Steine and Manager Virus Technology Karine Lindmo Yttredal, and will contribute with expertise and vaccine development in Dept. Prev Fish Health.

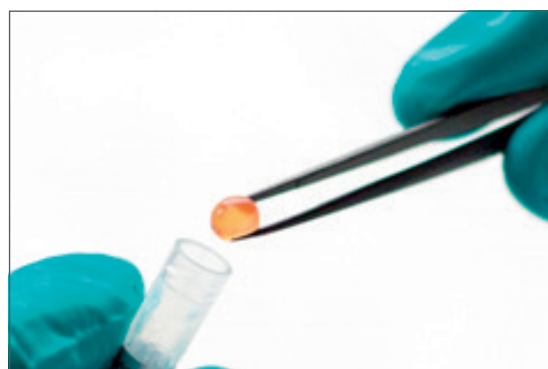


Figure 4.17. Pharmaq Analytiq use targeted research and effective analysis to ensure fish health and welfare in aquaculture (Photo: Pharmaq Analytiq)

5. Scientific activities and results

Common projects

DATABASE - Environmental and biological requirements & surveillance database for closed systems

*Project leader: Lars Ebbesson, UNI Research
R&D partners: Sigurd Handeland, Tom Ole Nilsen, Marco Vindas, Simon Mackenzie, Bendik Fyhn Terjesen, Jelena Kolarevic, Alexi Krasnov, Sven Martin Jørgensen, Eirik Thorsnes, Klaus Johannsen.*

Introduction

While knowledge of smolt production in traditional systems is extensive, there are major knowledge gaps about salmon and production in closed-containment systems (CCS). Research has mainly used low intensity

conditions, not resembling the CCS environment, and results and recommendations are therefore not directly useful for engineering and management of CCS. In order for Dept. of Tech and Env and user partners to design large-scale CCS for salmon production, more knowledge is urgently required on optimal range of water quality (e.g. O₂, CO₂, nitrate, oxidants, salinity, particles), hydrodynamics (e.g. swimming speed), fish density, temperature and light (Handeland et al., 2013 a and b). In CtrlAQUA we will run a series of small-scale experiments in RAS and flow-through, covering several size ranges (smolts, post-smolts and on-growing). The experiments will combine environmental and physiological data to determine environmental requirements for optimal performance and welfare of salmon in CCS. We will approach actual rearing situations and focus trials on water quality and hydrodynamic monitoring and how they affect fish performance metrics, welfare, and health. Task results will be compiled into a common database format that user partners can use for engineering or assessing biological performance. The aim of this project has been to establish a database that will assimilate environmental and biological requirements investigated in CtrlAQUA as well as a surveillance tool for closed and semi-closed systems. Sub-goals 2015: 1) To identify environmental and biological parameters to be included in the database, determine user frequencies of measurements per parameter, and identify additional parameters and/or frequencies needed to optimise the assessments. 2) Identify common database and routines that would facilitate both user and R&D partner input and use



Figur 5.1. CtrlAQUA technicians from Nofima measure flow rates through intake pipes to a large-scale salmon culture tank.

Results and Discussion

Parameter list. In order to assimilate all parameters into one database, we have divided

them into the physical environment, biological production and welfare, and health and disease. The database will be set up to receive a set of template files that are customised to upload data from User partner databases associated with field trials and experimental data from R&D partners. The frequency of individual data will be set by the experimental design with input from both User and R&D partners on a project basis with a minimum frequency.

Database identified. Together with Uni Computing, we have identified the open-source OpenTSDB database to be used in CtrlAQUA. This database is especially well suited for time-series analysis, the majority of CtrlAQUA data and analysis. Time-series are stored in OpenTSDB that is highly scalable and fault-tolerant. Data can be imported and exported in many ways, and with the templates that will be made for User and R&D partners, easy and robust uploading will secure few data errors. With the flexible plugin system we will be able to add a variety of search, analysis and graphing options. An additional advantage of OpenTSDB is that it will be used inside a big data cluster that includes Apache Hadoop, Apache Kafka, Apache Spark. Apache Spark has highly scalable machine-learning analysis tools for both real-time and historic data.

In 2016, we will also establish new features to retrieve and analyse data within the database. The main goal for 2016 will be to 1) ensure that the database is available and used by R&D Partners and 2) develop database protocols for retrieving relevant data analysis. In all large projects with many partners it is essential that we establish as often as possible standard routines in data collection and analysis. We aim to standardize as much as possible the data that will be put into the database to ensure proper assessment later on.

References

Handeland, S.O., Imsland, A.K., Ebbesson, L.O.E., Nilsen, T.O., Hosfeld, C.D., Baeeverfjord,

G., Espmark, Å., Rosten, T., Skilbrei, O.T., Hansen, T., Gunnarsson, G.S., Breck, O., Stefansson, S.O. 2013a. Low light intensity can reduce Atlantic salmon smolt quality. *Aquaculture* 384-387, 19-24

Handeland, S.O., Imsland, A.K., Ebbesson, L.O.E., Nilsen, T.O., Hosfeld, C.D., Teien, H.Ch., Stefansson, S.O. 2013b. Osmoregulation and growth in offspring of wild Atlantic salmon at different temperatures *Environmental Biology of Fishes*, DOI 10.1007/s10641-013-0151-5

BARRIER – Osmoregulatory barrier function in post-smolts reared in closed-containment systems

Project leader: Sven Martin Jørgensen, Nofima

R&D partners: Henrik Sundh, Tom Ole Nilsen, Kristina Sundell, Gerrit Timmerhaus, Aleksei Krasnov, Lene Sveen, Jelena Kolarevic, Bendik Fyhn Terjesen, Sigurd Stefansson, Sigurd Handeland, Lars Ebbesson.

Background

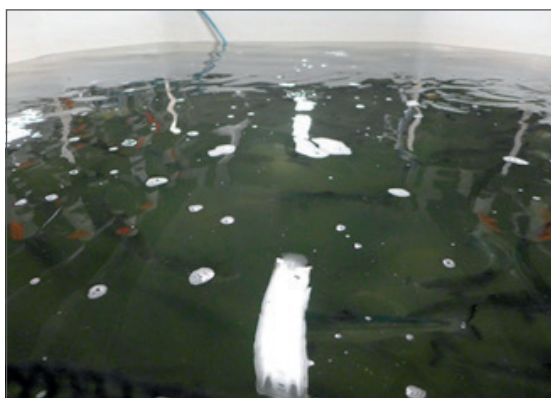
The expected higher production intensities of closed or semi-closed containment systems (CCS) may compromise skin health and lead to more efficient pathogen transmission and disease. Furthermore, transport of post-smolts to open sea cages may give osmoregulatory challenges and wound issues, with potential mortality and reduced welfare. Conservative estimates claims that 1-2.5% of farmed salmon dies from wounds. Skin mucosal surface drying out at salinities above 14 ppt is assumed to cause wounds and fin erosions (Takle et al. 2015). Recently we detected Na, K-ATPase expression in the skin suggesting an active osmoregulation (Nilsen et al., unpublished data). This contradicts the prevailing view that ionocytes are mainly expressed in skin at larval stage. The BARRIER project has performed add-on ex-

perimental activities to a large trial conducted in the RCN funded SalmoFutura project (Nofima and CtrlAQUA partners Uni Research and University of Bergen), in order to test the hypothesis that the barrier function of skin is influenced by osmoregulatory mechanisms, and whether different CCS strategies, salinities and acute or chronic stressors will affect skin barrier function and physiology of Atlantic salmon post-smolts.

Materials and Methods

Fish experiment

Experimental activities started on 19.05.2015 at Nofima Center for Recirculation in Aquaculture in Sunndalsøra (SalmoFutura project WP1 Task 2). Atlantic salmon post-smolts were reared in two types of closed-containment systems; RAS (simulating conditions in commercial land-based systems at 12 ppt salinity) and flow-through systems (FTS, simulating floating semi-closed containment systems in sea at full salinity). Each system included groups with low (LD; 25-35 kg/m³) and high (HD; 80-110 kg/m³) stocking densities, simulating respectively normal and chronic stress conditions. At three time points (S1=360 gr, S2= 560 gr, S3= 900 gr average body mass), all groups were subject to an acute stress test (ACT; crowding at 300 kg/m³ for 30 min, O₂ saturation > 50%) with organ sampling pre- (0 h) and 24 h post-stress.



Figur 5.2. Postsmolt salmon kept at high fish density in a RAS experimental tank. (Photo: Jelena Kolarevic)

Osmoregulatory capacity and ion transport

Effects of rearing systems and acute stress on active ion pumping and osmoregulatory capacity were analysed from the S1 stage, including gill NKA enzyme activity, plasma chloride and gene expression in skin tissue using qPCR assays for NKA- α 1b and NKCC1a.

Skin barrier integrity and physiology

The physiological properties of the skin were assessed using an Ussing chamber (Sundell and Sundh 2012) for measuring three electrical parameters that give information on the physiological function and barrier properties of the skin epithelium. In short, TER is mainly a measure of the paracellular permeability, and SCC describes the sum of active transports taking place in the apical and basolateral membranes of the enterocytes. The paracellular permeability of the skin epithelium was assessed as the apparent permeability (*P_{app}*), the diffusion rate (cm s⁻¹) of mannitol, a paracellular permeability marker. Selected skin samples were evaluated by immunohistochemical staining for expression of different ion transporter antibodies. Skin samples from S1, S2 and S3 stages were analysed.

Skin gene expression

Effects of rearing system, stocking density and acute stress on global molecular responses in skin were assessed by transcriptome analysis using the SIQ microarray (Krasnov et al. 2011) and bioinformatics system (STARS/ GeneHub). Skin samples from the S1 stage were analysed.

Results & Discussion

Osmoregulatory capacity and ion transport

Post-smolt reared in both RAS and FTS had adapted gill NKA enzyme activity levels close to expected when reared under 12 ppt and full-strength seawater, as was further indicated by the ability to maintain plasma chloride

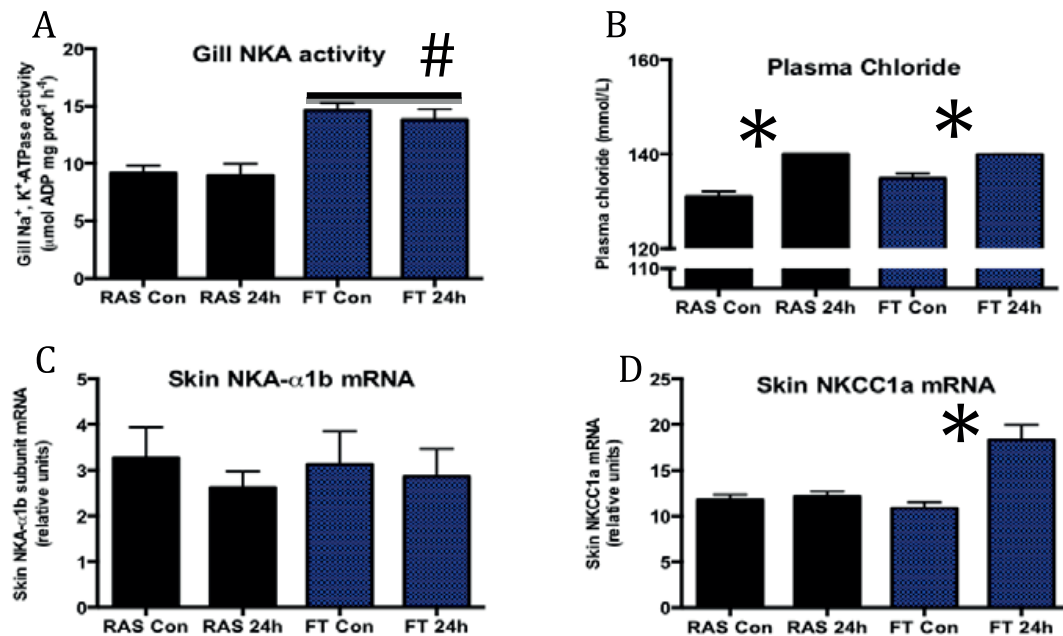


Figure 5.3. Gill NKA enzyme activity (A), circulating plasma chloride levels (B), expression of skin NKA- α 1b (C) and NKCC1a (D) mRNA levels in Atlantic salmon post-smolt reared in RAS and FT systems. Note that the used I-stat methodology only detects chloride levels up to 140 mmol/L and plasma chloride thus probably exceeds 140 mmol/L in 24h ACT post-smolts.

levels within a normal physiological range. Gene expression of NKA- α 1b and NKCC1a in skin suggested presence of active ion secretory cells (ionocytes) in post-smolts (Fig 5.3C,D). An increase in skin NKCC1a expression in ACT challenged post-smolt kept at FTS indicated some adaptive modulation in secretory capacity when plasma chloride levels are elevated. Preliminary results of immunostaining of NKA antibodies in skin using the T4 antibody resulted in immunoreactivity around the epithelial cells of the skin epidermis (data not shown). Preliminary results indicated no immunoreactivity by the α 5 antibody, known to cross-react to all α isoforms of the Atlantic salmon NKA (data not shown). However, using an antibody specifically directed towards NKA α 1c isoform resulted in low levels of immunoreactivity in the epidermal layer (data not shown).

Skin barrier integrity and physiology

Transepithelial resistance (TER) was in the same range for FTS and RAS, but increased significantly from S1 to S3 (for both LD and

HD), implying a tighter skin barrier with increasing post-smolt size (Fig 5.4A). In FTS, chronic stress (HD) lead to higher TER at S1, while there was a tendency ($p=0.063$) for the opposite at S3 (Fig 5.4A). In RAS, TER also increased from S1 to S2 (Fig 5.4B). Although not significant, the same tendency of lower TER in HD groups was observed at S2/S3 (Fig 5.4B). At S3, there was an overall reduction in TER after acute stress, but with no differences between densities. Hence, chronic and acute stress tends to increase skin permeability towards ions with potential consequence being increased cost for osmoregulation.

Paracellular permeability (P_{app}) for mannitol was similar between FTS and RAS at the different sampling points (Fig 5.5A,B). In the FTS fish, P_{app} decreased between S1 and S3, and HD fish displayed higher P_{app} compared to LD at S3 (Fig 5.5A). Also in RAS fish, the P_{app} decreased between S1 and S2 (Fig 5.5B), collectively suggesting tighter skin barrier with increased post-smolt growth size. P_{app} increased after acute stress in the LD group, but not in the HD group (Fig 5.5B). A

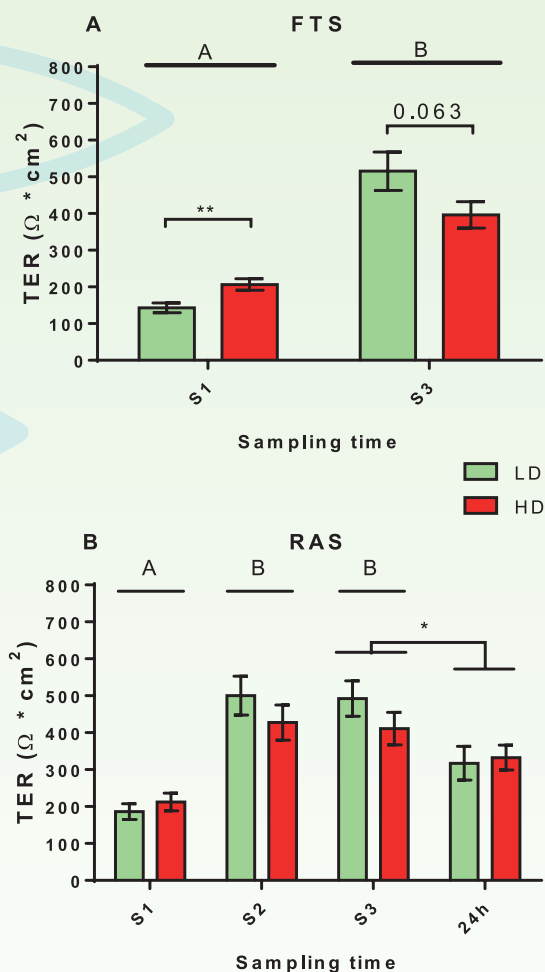


Figure 5.4. Transepithelial resistance (TER) measurements of skin tissue between FTS and RAS reared post-smolts at S1-S3 samplings, including chronic stress (density; HD vs LD) and 24 h after acute crowding stress test.

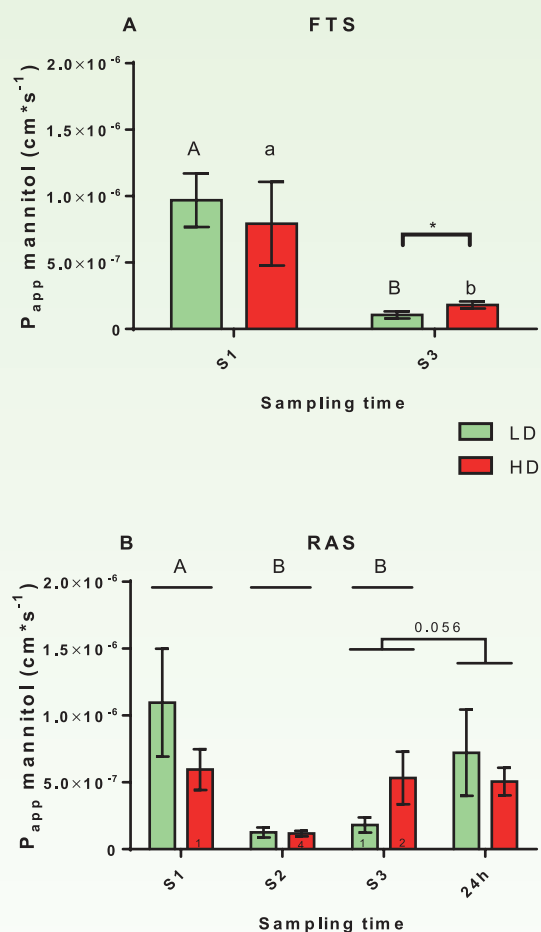


Figure 5.5. Paracellular permeability (Papp) for mannitol in skin tissue between FTS and RAS reared post-smolts at S1-S3 samplings, including chronic stress (density; HD vs LD) and 24 h after acute crowding stress test.

correlation test aiming to evaluate potential relationship between the two barrier function measurements (TER and Papp) revealed a significant correlation between TER and Papp on sampling S1, S3 and after acute stress. In conclusion, chronic and acute stress tends to increase skin permeability towards small molecules with potential consequence of increased disease susceptibility.

Gene expression analysis of skin

Microarray analyses revealed significant changes in skin gene expression between post-smolts reared in FTS versus RAS, while

the effect of density in each system was weaker. The major changes in gene expression occurred 24 h after acute stress challenge, and the magnitude of expression changes were significantly higher in groups reared at full salinity in FTS compared to 12 ppt RAS. Responses suggested a compensation to a severe cellular and extracellular stress condition, evidenced by upregulation of stress responses (heat shock proteins, redox status and stress activated kinases), extracellular matrix, cytoskeleton, inflammation, immune-IFN and a wide range of metabolic responses. The stronger induction in skin of FTS fish suggests increased cost of acclima-

tion and recovery from the severe crowding stress.

References

Krasnov, A., Timmerhaus, G., Afanasyev, S., Jørgensen, S.M. 2011 Development and assessment of oligonucleotide microarrays for Atlantic salmon (*Salmo salar* L.). *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics* 2011, 6(1), 31-8

Sundell, K.S., Sundh, H. 2012. Intestinal fluid absorption in anadromous salmonids: importance of tight junctions and aquaporins. *Front. Physio.* 3, 388. Doi: 10.3389/fphys.2012.00388

Takle, H., Ytteborg, E., Vale Nielsen, K., Karlsen, C.R., Nilsen, H., Sveen, L., Colquhoun, D., Olsen, A.B., Sørum, H., Nilsen, A. 2015. Sårproblematikk og hudhelse i laks- og regnbueørretoppsett. *Nofima Rapport* 5/2015.

SENSOR – Sensor protection and maintenance in closed systems

Project leader: Øyvind Mikkelsen, NTNU
R&D partner: Bendik Fyhn Terjesen

Background

The project SENSOR works on developing protection systems for sensors, and improved sensor systems for water quality monitoring in closed-containment systems (CCS). To know the water quality and any changes in it is of outmost importance for the fish health, and to secure optimal conditions for the production. Water quality (WQ) monitoring is an integrative part of closed containment system management. However, the current sensor technologies are in many cases not adequate for CCS. A severe problem is formation of biofilms and siltation of the sensor surfaces after some time use, which typically results in changes in the sensor response and incorrect

readings (Delauney et al., 2012; Kolarevic et al., 2011). Many sensors also need special attention for maintenance and area of use could be restricted to specific pH ranges etc. One example is that many sensors contain internal solutions, which might change over some time, resulting in significant drift and changes in signal (Zuliani et al., 2012). Issues like this in addition to several other types of possible errors demand for improved sensor systems, independent of whether the sensor principles are based on electroanalytical, optical or other types of techniques. Protections and optimized maintenance procedures are important steps in this area. In some cases also new type of sensors needs to be developed (Mikkelsen et al., 2006).

The SENSOR project works on design, construction and testing of different types of protecting systems, to achieve improved stability and long-time reliability of sensor systems for water quality in CCS. Both optimized maintenance routines using combinations of chemical, manual and electrochemical cleaning procedures will be tested and implemented. Further, several types of protecting grids and stockings will be designed, constructed and tested. Typical sensor housing materials (and real sensors) will be exposed and imbedded in authentic CCS water or similar solutions, and the growing of biofilm and siltation will be observed over time for systems without and with different combinations of maintenance / cleaning procedures and protecting technologies. In a long-time perspective of the SENSOR project, the goal is to develop improved systems for water quality measurement, especially for CO₂, pH, NO₃⁻, and selected trace elements (e.g. Cu, Fe).

This contribution will report results and achievements so far in the SENSOR project, which includes selections of protecting systems for sensors in CCS and preliminary results from automatic system for trace metal monitoring.

Materials and Methods

This section will be divided into two parts. First part will be a short overview (review) of selected materials for protection systems for sensors installed in CCS, and the second part will be a short description of preliminary tests of an automatized system for determination of copper for use in CCS.

Materials for protection systems

The idea behind the protection system within the SENSOR project is to develop easy to use protection grids / housing or stockings that can be placed outside commercially available sensor, with the intention to increase the stability over time and reduce the frequency of maintenance. A main issue connected to sensors in seawater and in general is formation of biofilms (Strathmann et al., 2013). To avoid biofouling it is therefore important to use materials that are growth-inhibiting for microorganisms (Pelletier et al., 2009) especially in marine water or brackish water.

To achieve sensor protection systems that will be practically easy to use and to handle by personnel in CCS, combinations of a typical stocking / housing material with a growth

inhibiting component is required. Typical materials suitable for construction of the stocking to cover the sensors are synthetic fibres like nylon or plastic materials typically used for e.g. protection of underwater cameras (McLean et al., 2015). Other suitable covering materials are cellulose film, polyamide, fiberglass, polyethersulfone and silica based material. Typical growth growth-inhibiting compounds could be zinc and copper based compounds and paints (Karlsson and Eklund, 2004), silver and silver compounds (Nguyen and Roddick, 2012) or alloys of such compounds. Based on criteria mention above materials for constructing protection grids / housing or stockings are given in table 5.1.

The systems given in Table 5.1, will also be tested in combination with electrochemical cleaning using platinum wires for generating chlorine locally over the sensor surface by electrolysis of sea water (Kraft et al., 1999) to maintain a clean surface. To avoid deposits of particles and sludge small step engines will be mounted on the sensors to achieve a oscillation for removal of particles etc. Different frequencies will be tested.

Table 5.1. Selected materials for sensor protection grids / housing or stockings

Grid / Housing / Stocking material	Growth-inhibiting compound
Synthetic textile fibres (e.g. polyamides)	Coated with or mixed with the strands of metals such as copper, silver or other metals/alloys (copper-zinc) or paints with such compounds
Carbon nanotubes	Coated with metal as copper, silver or other metals/alloys or paints containing such compounds
Polyamide	Fibres coated with silver, polyamide/silver composites
Polyethersulfone	Polyethersulfone nanocomposites with imbedded copper
Polytetrafluoroethylene	Incorporated copper, silver or other metals/alloys
Fiberglass	Combined with antifouling paints
Nafion (film)	Combined with copper wires / grids
Cellulose (film)	Aerogel-Based with silver nano particles
Silica	Doped with copper / copper compounds

Preliminary tests for automatized system for determination of copper

Copper is an important compound which might directly affect the fish health if present in the water in CCS. The optimum copper concentration is relatively narrow, and both too low and too high concentrates will have negative effect. Copper is of especially concern in recirculating aquaculture systems as this element often is added as a supplement in the fish meal diet feed. Therefore, it is of great importance to monitor copper concentration in such systems (Lorenzen et al., 1998).

System for automatized monitoring of copper (and other possible nutrients and trace elements) is shown in Figure 5.6a. The system used includes a computer and a dosage pump for addition of needed chemicals, which in this experiment was HCl (1 M) mixed to 0.01 M in the final measuring sample. The measuring principle, based on voltammetry, uses nontoxic sensors, which is included in a cell system shown in Figure 5.6b. To the system, there is also connected two pumps, one for filling and one for emptying the measuring cell. Additionally a potentiostat to carry out the measuring of the samples are con-

nected, and controlled by a remote operating software.

Results

Figure 5.7 shows results from a 60 days test run of the automatic system for copper determination in water for smolt production. Water samples taken by the automatic sampling system, was then immediately measured by the installed sensors. Measuring rate was one recording pr. hour. There was no maintenance carried out on the system during the period. For testing the reliability of the system, a known amount of copper (5 ppb) was added at day 25. As seen in figure 5.7, this addition of copper was identified by the system. Average concentration of copper in that measuring period was $1.3 \mu\text{g/l}$, the maximum and minimum concentrations was 2.3 and $0.5 \mu\text{g/l}$ respectively.

Conclusions

Based on the work so far, the next step will be to test the different sensor protection systems under real conditions in closed-containment aquaculture systems as well as under controlled laboratory conditions.

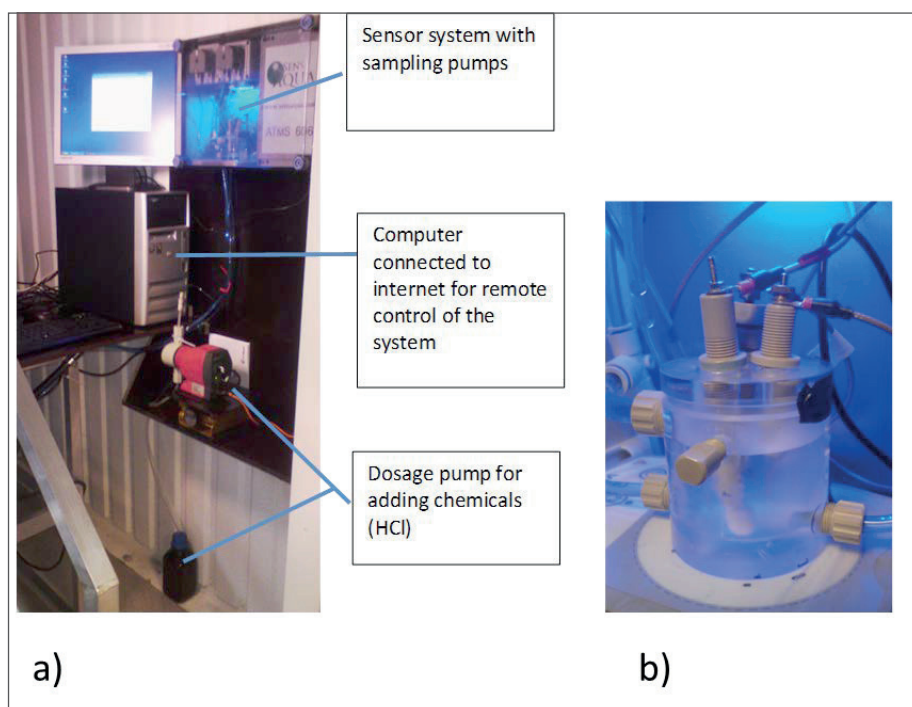


Figure 5.6. a) Setup of measuring system, and b) sensor cell system

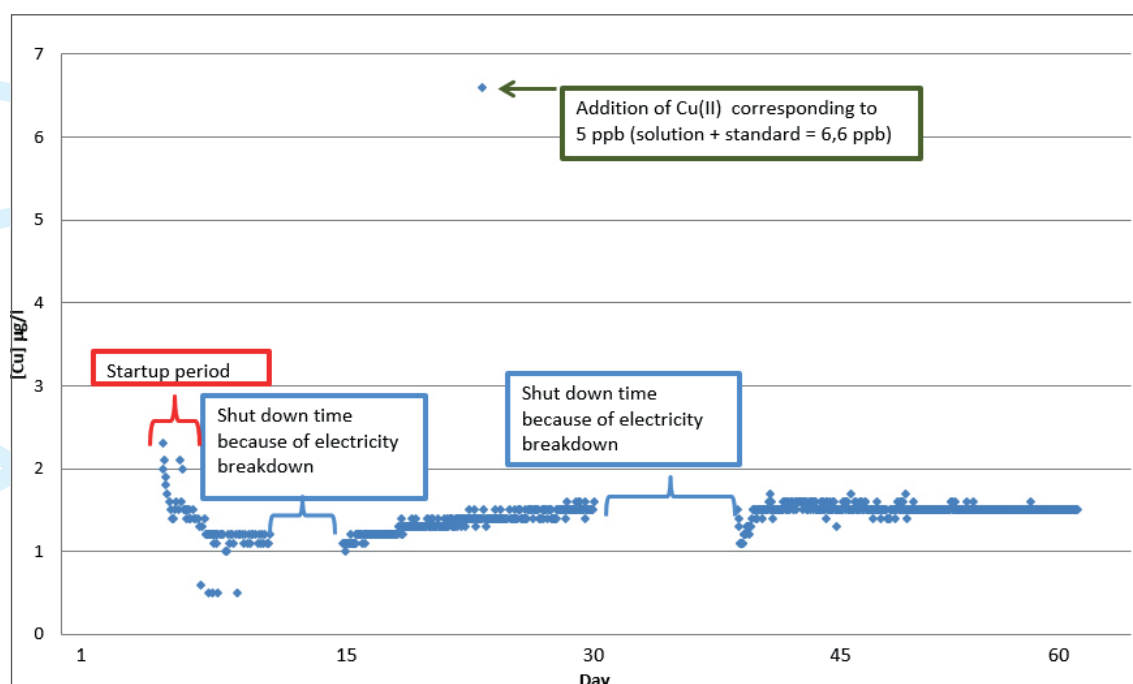


Figure 5.7. Measurement of copper in water samples at a smolt production plant taken every hour.

Based on the preliminary tests of the sensor system for automatized copper determination the results indicate that concentration of copper in relevant water samples from CCS can be monitored continuously with satisfactory results. Two electricity breakdowns of some days duration did not affect the further measurements. It is assumed that the system might operate up to two month without manual attendance, however this will depend on the water quality. The setup and used chemicals was especially designed for copper determination, but other nutrient and trace metals will also be able to monitor with this technique.

References

Delauney, L., Compère, C., Lehaitre, M., 2010. Biofouling protection for marine environmental sensors, *Ocean Science* 6, 503-511

Karlsson, J., Eklund, B., 2004. New biocide-free anti-fouling paints are toxic, *Marine Pollution Bulletin* 49, 456-464

Kolarević, J., Cirić, M., Zühlke, A., Terjesen, B.F., 2011. On-line pH measurements in recir-

culating aquaculture systems (RAS), *Aquaculture Europe 2011. European Aquaculture Society, Rhodes, Greece*, 570-571.

Kraft, A., Stadelmann, M., Blaschke, M., Kreysig, D., Sandt, B., Schröder, F., Rennau, J., 1999. Electrochemical water disinfection Part I: Hypochlorite production from very dilute chloride solutions. *Journal of Applied Electrochemistry* 29, 859-866

Lorentzen, M., Maafe, A., Julshamn, K. 1998. Supplementing copper to a fish meal based diet fed to Atlantic salmon parr affects liver copper and selenium concentrations, *Aquaculture Nutrition* 4, 67-72

McLean, D.L., Green, M., Harvey, E.S., Williams, A., Daley, R., Graham, K.J. 2015. Comparison of baited longlines and baited underwater cameras for assessing the composition of continental slope deepwater fish assemblages off southeast Australia, *Deep Sea Research Part I: Oceanographic Research Papers* 98, 10-20

Mikkelsen, Ø., van den Berg, C.M.G., Schröder,

H.H., 2006. *Determination of Labile Iron at Low nmol L⁻¹ Levels in Estuarine and Coastal Waters by Anodic Stripping Voltammetry, Electroanalysis* 18, 35-43

Nguyen, T., Roddick, F.A., Fan, L. 2012. *Biofouling of Water Treatment Membranes: A Review of the Underlying Causes, Monitoring Techniques and Control Measures, Membranes* 2, 804-840

Pelletier, E., Bonnet, C., Lemarchand, K. 2009. *Biofouling Growth in Cold Estuarine Waters and Evaluation of Some Chitosan and Copper Anti-Fouling Paints, International Journal of Molecular Sciences* 10, 3209-3223

Strathmann, M., Mittenzwey, K.H., Sinn, G., Papadakis, W., Flemming, H.C. 2013. *Simultaneous monitoring of biofilm growth, microbial activity, and inorganic deposits on surfaces with an in situ, online, real-time, non-destructive, optical sensor, Biofouling* 29, 573-583

Zuliani, C., Diamond, D., 2012. *Opportunities and challenges of using ion-selective electrodes in environmental monitoring and wearable sensors, Electrochimica Acta*, 84, 29-34

RISK - Review of the microparasites that could represent a future problem for production of salmonids in closed or semi-closed containment systems

Project leader: Are Nylund, UiB

R&D partners: Christian R Karlsen, Chris Good, Svein M. Jørgensen, Heidrun Plarre, Trond E. Isaksen, Sigurd O. Handeland, Knut Wollseth, Karl F. Ottem

Background

Norway has been a leading country in the development of new technologies for production of Atlantic salmon. The transition from double rows of steel cages, separated

from each other by just a meter or so, to the larger polar rings increased the water quality within the nets and has allowed the production of larger amounts of salmon at each site. This transition has possibly also reduced the transmission of microparasites between cages within a farming site. Still, open production will always be vulnerable to infection by parasites with long living transmission stages (spores and free-living larvae). It has been suggested that the next step forward should include closed- or semi-closed containment systems (CCS or S-CCS) (Vilinn Tolås 2012). Smolt production in fresh water has already established technologies for recirculation of production water, Recirculating Aquaculture Systems - RAS. The use of S-CCS in the marine production phase of salmon makes it possible to prevent or reduce the influx of macroparasites by stocking pathogen-free smolt and controlling the intake water (cf. Vilinn Tolås 2012). However, the weakness of these systems is that it must be expected that introduction of microparasites, which can spread by direct transmission, could pose a major threat for the future success of these more environmental-friendly systems. Hence, response strategies for handling disease problems in S-CCS will have to be developed. S-CCS should reduce the number of farmed salmon populations that will become infected by microparasites, but they should also reduce the importance of farmed populations as reservoirs for microparasites if the effluent water from these systems is treated.

This is a short, updated, summary of existing knowledge about selected microparasites that could represent a future problem for production of salmonids in CCS.

Results

Viruses

Several viruses are influencing the production of salmonids in Norway. The first viruses that were recognized as problems in Norwegian salmon farming were *Infectious*

pancreatic necrosis virus (IPNV), isolated from Norwegian salmon in 1976 (Håstein & Krogsrud 1976), followed by *Infectious salmon anaemia* (ISA virus) in 1984 (Thorud & Djupvik 1988, Hovland et al 1994, Dannevig et al 1995) and pancreas disease, PD (Salmonid Alphavirus) in 1988 (Poppe et al 1989, Nelson et al 1995, Christie et al 1998, Nylund et al 2003, Hodneland et al 2005). During the last decade a range of new viral diseases and viruses (Atlantic salmon paramyxovirus ASPV, Salmonid gill poxvirus SGPV, Piscine orthoreovirus PRV, Piscine myocarditis virus PMCV, Atlantic salmon calicivirus ASCV) have emerged in Norwegian salmon farms (Nylund et al 2008a, Palacios et al 2010, Haugland et al 2011, Mikalsen et al 2014), but among these only ASPV can be cultured in available cell cultures (Kvellestad et al 2003, 2005, Fridell et al 2004, Nylund et al 2008b). All these viruses have a direct transmission and could become important risk factors in S-CCS.

Bacteria

In the first decades of Norwegian salmon farming until the early 90's, one of the major challenges was bacterial infections. Diseases such as vibriosis caused by *Vibrio* (*Listonella*) *anguillarum* (Egidius & Andersen 1978), cold water vibriosis caused by *Aliivibrio salmonicida* (Egidius et al 1986), furunculosis caused by *Aeromonas salmonicida* ssp. *salmonicida* and atypical furunculosis caused by atypical *Aeromonas salmonicida* (Austin & Austin 2007) resulted in significant losses. Today, vaccines are effectively restraining outbreaks of these diseases in Atlantic salmon. Other bacteria like *Renibacterium salmoninarum* are under control due to screening of brood fish and removal of positive fish from the production line.

The bacteria that still represent problems for the salmon industry in Norway are *Flavobacterium psychrophilum* and *Yersinia ruckeri* in fresh water and *Moritella viscosa*, *Tenacibaculum* spp., and *Y. ruckeri* in sea water. Both *M. viscosa* and *Tenacibaculum* spp may also

become a problem at smolt production sites when sea water intake is used. Gill diseases are a major problem in marine salmon production in western and middle Norway and several intracellular bacteria are associated with this problem. These are represented by two *Chlamydiaceae*, *Candidatus* *Piscichlamydia salmonis* (both fresh and sea water), *Candidatus* *Syngnamydia salmonis*, and the β -proteobacterium *Candidatus* *Branchiomonas cysticola* (both fresh and sea water). An additional chlamydia species, *Candidatus* *Clavichlamydia salmonicola*, occur on the gills of salmonids in fresh water (Karlsen et al 2008). All these intracellular bacteria are associated with epitheliocystis (Nylund et al 1998). Norwegian smolt production will increasingly occur in recirculating aquaculture systems in closed containment facilities in either land-based recirculation farms or semi-closed floating farms. Initial production have so far not been hampered significantly by any particular health challenges, although outbreaks of yersiniosis have resulted in losses, and outbreaks of winter-ulcer disease associated with *M. viscosa*, *A. wodanis* and *Tenacibaculum* spp. isolation are reported in production facilities utilising seawater (Bornø & Linaker 2015). However, production is relative small-scaled and intensive production in S-CCS is expected to increase problems related to risk of bacterial diseases, ulcer development and fin-injuries.

Parasites

Parasites constitute a serious problem in the production of Atlantic salmon in Norway. Two of these, the salmon louse *Lepeophtheirus salmonis* and the tapeworm *Eubothrium* sp. have been associated with salmon production from the early beginning. The former has been considered as the largest problem in the salmon industry during the last two decades, while the tapeworm has, to a large extent, been ignored even though it reduces growth of the salmon and increases the production costs (Saksvik et al 2001ab). It is believed that S-CCS, if the sea water intake is below the

plankton-layer, will reduce the impact of both these macro-parasites, and this seem to be the case for the salmon louse in preliminary tests (Villin Tolås 2012). The two macro-parasites mentioned have both complex life cycles, the salmon louse with three free-living stages and the tapeworm with an intermediate host (copepod), while the microparasites like *Ichthyobodo* spp. and *Paramoeba perurans*, have direct life cycles with transmission directly between individual salmon (Isaksen 2013, Crosbie et al 2012). This means that once introduced to S-CCS the protozoans will have the potential to become a serious problem.

Fungi

A range of different species of fungi are associated with fish diseases. Most of these are present in fresh water only, but diseases associated with *Exophiala* species have also been detected in sea water production of Atlantic salmon. However, disease associated with members of *Exophiala* is not considered as an important problem in salmon farming in Norway. Fungal diseases of salmonids in fresh water are usually associated with the surface areas of the host and the causal agent is in most cases a member of the genus *Saprolegnia*. Members of this genus are quite common in the fresh water environment and contribute to downgrading of dead organic material. This suggests that in most cases they are secondary invaders attacking hosts weakened by other causes. However, they can be primary pathogens attacking eggs/embryos in addition to larvae, fry, par and smolt. Important preventive measures include removal of dead organic material and securing a good water quality optimal for the salmonid hosts. No vaccines are available. The following strategies have been used as control measures against saprolegniasis; hydrogen peroxide, formalin, sodium chloride (sea water), bronopol, copper sulphate, UV radiation and ozone treatment.

The most important parasitic member of the fungi attacking salmonids in sea water in Nor-

way is the microsporidian *Paranucleospora theridion* (syn *Desmozoon lepeophtheirii*) (Nylund et al 2010). It belongs to a large group of fish parasites that previously was classified as protozoans, while more recent genetic studies have shown that they belong to the fungi. *P. theridion* has a complex life cycle with two developmental stages in the Atlantic salmon. The final host for this parasite is the salmon louse. It is expected that the use of S-CCS should reduce the impact of *P. theridion*.

Literature

Austin, B., Austin, D.A. 2007. *Bacterial Fish Pathogens: Disease of Farmed and Wild Fish*, 4 Edition. Springer Praxis, Chichester, UK.

Bornø, G., Linaker, M.L. 2015. *Fiskehelserapporten 2014*. Veterinary Institute, Harstad, Norway.

Christie, K.E., Fyrand, K., Holtet, L., Rowley, H.M. 1998a. Isolation of pancreas disease virus from farmed Atlantic salmon, *Salmo salar* L., in Norway. *Journal of Fish Diseases* 21, 391-394.

Crosbie, P.B.B., Bridle, A.R., Cadoret, K., Nowak, B.F. 2012. In vitro cultured *Neoparamoeba perurans* causes amoebic gill disease in Atlantic salmon and fulfils Koch's postulates. *International Journal for Parasitology* 42, 511-515.

Dannevig, B.H., Falk, K., Namork, E., 1995. Isolation of the causal virus of infectious salmon anemia (ISA) in a long-term cellline from Atlantic salmon head kidney. *Journal of General Virology* 76, 1353-1359.

Egidius, E., Wiik, R., Andersen, K., Hoff, K.A., Hjeltne, B. 1986. *Vibrio salmonicida* sp. nov., a new fish pathogen. *International Journal of Systematic and Evolutionary Microbiology* 36, 518-520.

Egidius, E.C., Andersen, K. (1978). Host-specific

ic pathogenicity of strains of *Vibrio anguillarum* isolated from rainbow trout *Salmo gairdneri* Richardson and saithe *Pollachius virens* (L.). *Journal of Fish Diseases* 1, 45-50.

Fridell, F., Devold, M., Nylund, A. 2004. Phylogenetic position of a paramyxovirus from Atlantic salmon *Salmo salar*. *Dis Aquat org* 59, 11 - 15.

Haugland, O., Mikalsen, A.B., Nilsen, P., Lindmo, K., Thu, B.J., Eliassen, T.M., Roos, N., Rode, M., Evensen, O. 2011 Cardiomyopathy syndrome of Atlantic salmon (*Salmo salar* L.) is caused by a dsRNA virus of the Totiviridae family. *J Virol* 85, 5275-5286,

Hodneland, K., Brattland, A., Christie, E., Endresen, C., Nylund, A. 2005. A new subtype of salmonid Alphavirus, *Togaviridae*, from Atlantic salmon *Salmo salar* and rainbow trout *Oncorhynchus mykiss* in Norway. *Dis Aquat Org* 66, 113 - 120.

Hovland, T., Nylund, A., Watanabe, K., Endresen, C. 1994. Observation of infectious salmon anaemia virus in Atlantic salmon, *Salmo salar* L. *J Fish Dis* 17, 291 - 296.

Håstein, T. Krogsrud, J. 1976. Infectious pancreatic necrosis, first isolation of virus from fish in Norway. *Acta Veterinaria Scandinavica* 176, 109-111.

Isaksen, T.E. 2013. *Ichthyobodo* infections on farmed and wild fish. -Methods for detection and identification of *Ichthyobodo* spp. Dr. thesis, University of Bergen, Norway (ISBN: 978-82-308-2201-2).

Karlsen, M., Nylund, A., Watanabe, K., Helvik, J.V., Nylund, S., Plarre, H. 2008. Characterization of *Candidatus Clavochlamydia salmonicola*: an intracellular bacterium infecting salmonid fish. *Environ Microbiol* 10, 208 - 218.

Kvellestad, A., Dannevig, B.H., Falk, K. 2003. Isolation and partial characterization of a

novel paramyxovirus from the gills of diseased seawater reared Atlantic salmon (*Salmo salar* L.). *J Gen Virol* 84, 2179 - 2189.

Kvellestad, A., Falk, K., Nygaard, S.M.R., Flesjå, K., Holm, J.A. 2005. Atlantic salmon paramyxovirus (ASPV) infection contributes to proliferative gill inflammation (PGI) in seawater-reared *Salmo salar*. *Dis Aquat Org* 67, 47 - 54.

Mikalsen, A.B., Nilsen, P., Frøystad-Saugen, M., Lindmo, K., Eliassen, T.M. 2014. Characterization of a Novel Calicivirus Causing Systemic Infection in Atlantic Salmon (*Salmo salar* L.): Proposal for a New Genus of Caliciviridae. *PLoS ONE* 9(9): e107132. doi:10.1371/journal.pone.0107132.

Nelson, R.T., McLoughlin, M.F., Rowley, H.M., Platten, M.A., McCormick, J.I. 1995. Isolation of a toga-like virus from farmed Atlantic salmon *Salmo salar* with pancreas disease. *Dis Aquat Organ* 22, 25-32.

Nylund, A., Kvenseth, A.M., Isdal, E. 1998. A morphological study of the epitheliocystis agent in farmed Atlantic salmon. *J Aquat Anim Health* 10, 43-55.

Nylund, A., Devold, M., Plarre, H., Isdal, E., Aarseth, M. 2003. Emergence and maintenance of infectious salmon anemia virus (ISAV) in Europe: a new hypothesis. *Dis Aquat Org* 56, 11 - 24.

Nylund, S., Nylund, A., Karlsen, M. 2008b. The complete genome sequence of the Atlantic salmon paramyxovirus (ASPV). *Virology Mar* 30;373, 137- 48. Epub 2007 Dec 21.

Nylund, A., Watanabe, K., Nylund, S., Karlsen, M., Sæther, P.A., Arnesen, C.E., Karlsbakk, E. 2008a. Morphogenesis of Salmonid Gill poxvirus (SGPV) associated with proliferative gill disease (PGD) in farmed Atlantic salmon (*Salmo salar*) in Norway. *Arch Virol* 153, 1299 - 309.

Nylund, S., Nylund, A., Watanabe, K., Arnesen, C.E., Karlsbakk, E. 2010. *Paranucleospora theridion* n.gen., n.sp. (Microsporidia, Entero-cytozoonidae) with a life cycle in the salmon louse (*Lepeophtheirus salmonis*, Copepoda) and Atlantic salmon (*Salmo salar*). *J Euk Micr* 57, 95-114

Palacios, G., Lovoll, M., Tengs, T., Hornig, M., Hutchison, S., Hui, J., Kongtorp, R.T., Savji, N., Bussetti, A.V., Solovyov, A., Kristoffersen, A.B., Celone, C., Street, C., Trifonov, V., Hirschberg, D.L., Rabadan, R., Egholm, M., Rimstad, E., Lipkin, W.I. 2010 Heart and skeletal muscle inflammation of farmed salmon is associated with infection with a novel reovirus. *PLoS One* 5:e11487

Poppe, T., Rimstad, E., Hyllseth, B. 1989. Pancreas disease in Atlantic salmon (*Salmo salar*) postsmolts infected with infectious pancreatic necrosis virus (IPNV). *Bulletin of European Associated Fish Pathologists* 9, 83-85.

Saksvik, M., Nilsen, F., Nylund, A. & Berland, B. 2001. Effect of marine *Eubothrium* sp. (Cestoda: Pseudophyllidea) on the growth of Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* 24, 111 – 119.

Saksvik, M., Nylund, A., Nilsen, F. & Hodneland, K. 2001. Experimental infection of Atlantic salmon (*Salmo salar* L.) with marine *Eubothrium* sp. (Cestoda: Pseudophyllidea): observations on the life cycle, aspects of development and growth of the parasite. *Folia Parasitologica* 48, 118 – 126.

Vilinn Tolås, I. 2012. Lukket merdsystem – AquaDomen: Effekt på smittedynamikk. Master thesis, Department of Biology, University of Bergen. pp 72

Thorud, K., Djupvik, H.O. 1988. Infectious anaemia in Atlantic salmon (*Salmo salar* L.). *Bull. Eur Ass Fish Pathol* 8, 109-111.

HYDRO - Hydrodynamic challenges in huge tanks (1000+ m³)

Project leader: Steven Summerfelt, Freshwater Institute

R&D partners: Bendik Fyhn Terjesen, Astrid Holan Buran, Frode Mathisen, Brian Vinci, Jagan Gorle

Background

To characterize the current status of large culture tanks in Norwegian industry, CtrlAQ-UA industry partners were surveyed to identify the availability of circular tanks larger than 400 m³ and characterize their existing operational parameters. This survey is part 1 of the HYDRO work plan. In part 2 of the HYDRO work plan, water rotational velocities and tank mixing data are being collected from several of the tanks identified in Part 1. The first such data are summarised in this report in order to highlight what will be the focus of Part 2. Part 3 of the HYDRO project will be to develop a computational fluid. Dynamics (CFD) model of a near 1000 m³ tank operated under base-line conditions, as suggested by this survey, and then verify that the model is calibrated by comparison with empirical data collected from such a tank. Once calibrated, the CFD model will be used to determine how variables such as splitting of flow to the upper and lower dual-drains, inlet nozzle velocities, and the tank hydraulic retention time impact water rotational velocities and mixing.

Materials and Methods

A survey was conducted (HYDRO Part 1) to determine the geometry, operating parameters, and other key features of large circular or octagonal culture tanks used to produce Atlantic salmon smolt and post-smolt at six major Norwegian Atlantic salmon production companies. The survey was limited to the following CtrlAQUA industry partners in Norway: Marine Harvest, Grieg Seafood, Cermaq,



Figure 5.8. Tanks at CtrlAQUA partner site used for survey of operational parameters in the HYDRO project

Lerøy Seafood, Njord Salmon, and Bremnes Seashore. In addition, the first empirical data (HYDRO Part 2) were collected across a vertical-profile in a 14.5 m diameter octagonal culture tank installed at one of the partner's smolt and postsmolt production sites. An engineer specialized in computational fluid dynamics was hired by Nofima (Sunndalsøra) to model the hydraulics in large culture tanks (HYDRO Part 3) and the benchmarking efforts, in this regard, were completed.

Results

The mean culture tank volume ranged from 500 to 1300 m³ per tank (21,000 m³ for a floating fiberglass tank in sea). Tank diameters ranged from 14.5 to 20 m diameter (40 m at the floating tank); some were octagonal tanks (Figure 5.8) but most were circular in design. Maximum tank depths ranged from 3.5 to 4.5 m, which produced diameter-to-average-depth ratios of 3.6:1 to 5.5:1 m:m. The floating tank was much deeper at 20 m, with a diameter-to-average-depth ratio of only 2.4:1 m:m. All tanks had sloped floors toward the tank center, with the tank center deeper than the tank wall by 0.3 to 0.65 m, i.e., a slope ranging from 4.0 to 6.5%. The floating tank in sea had a much stronger mean slope

(approximately 30%) to the bottom-center drain.

Water flow through each large culture tank ranged from 3 to 19 m³/min (400 m³/min at the floating tank), with an adjustable flow-rate reported at most facilities. The mean hydraulic retention time (HRT) at maximum reported flow ranged from 35 to 170 minutes. Interestingly, about half of the large tank construction or renovation projects have taken place since 2013, and the more recent tank construction/ renovations are operated with much more rapid tank flushing rates, i.e., from 34.8 to 52.5 minute HRT. Large tanks built before 2013 were operated at a HRT of 67 to 170 minute.

Maximum feed load on each of the land-based tanks ranged from 525 to 850 kg/day, but reached 3,700 kg/day at the floating tank. Feed load did not correlate with flow rate through the same tank. Yet, the three tanks with the highest tank flow rate (greater than 17.6 m³/min) were all built since 2013. Whereas, the tanks with the least flow rate (< 12 m³/min) began operating before 2011.

Maximum biomass densities ranged from 40 to 70 kg/m³ at the land-based facilities, but

were only 20 kg/m³ at the floating tank.

Fewer than half of the tanks operated dual drains. Dual-drain tanks use either an elevated drain at tank center or sidewall. In nearly all cases of those tanks surveyed here, most of the flow was discharged through the bottom-center drain of the dual-drain tank. The exception was the floating tank, which operated with only 20% flow through the bottom-center drain, and the remainder through side-wall drains located almost at the bottom of the tank.

The culture volumes always contain sloping floors and vertical posts (to support walkways) and/or piping to flush dead fish or carry water away from the bottom drain, that create drag and reduce tank rotation and possibly negatively impact mixing, particularly close to the center of the tank.

Empirical data (collected in one of the 14.5 m diameter octagonal culture tanks installed at one of the partners smolt and postsmolt production sites) indicate that dissolved oxygen ranged from 90% saturation to just under 80% saturation, and suggest that mixing at the middle of the tank was reduced. In addition, the water rotational velocities across a vertical-profile of the culture tank indicate that water rotation ranged from 18-30 cm/s (data not shown). At the time of sampling, the culture tank contained 370,000 fish at a mean weight of approximately 36.5 g and a biomass density of 17 kg/m³. Inlet water carried 114% oxygen saturation into the tank. Total water flow through both inlet supply pipes was measured at 13.5 m³/min, which provided a mean hydraulic retention time of 58 minutes.

Discussion

This large tank survey highlights the prevalence (55) of large (500 to 1300 m³ per tank) land-based circular-type culture tanks (along with 1 floating tank) and a recent trend

towards an increased awareness of limits on metabolic waste accumulation and general fish welfare in Norwegian land-based Atlantic salmon smolt and post-smolt facilities of the project partners. Of note, tanks installed or renovated since 2013 are operated at mean tank HRT's of 35 to 50 minutes (compared to tank HRT's of 67 to 171 minutes in the previous years) and can support higher feed loading rates and/or be used to improve flushing of waste metabolites and prevent water quality (particularly elevated dissolved CO₂) that compromises salmon performance and welfare. Additionally, the culture tank flow per unit of feed load in land-based tanks that began operating before 2010 were lower (19-30 m³/day flow per kg/day feed = 19-30 m³ flow/kg feed), i.e., more intensely operated, than in tanks that began operating later (33-40 m³ flow/kg feed).

From a metabolic standpoint, the maximum cumulative feed burden on the culture tanks built before 2010 would consume approximately 12 to 21 mg/L of oxygen in a single pass across the culture tank, assuming that 0.35-0.40 kg of oxygen are consumed by swimming fish for every kilogram of feed consumed. In contrast, land-based tanks built/retrofit more recently would require 8.8-12 mg/L of oxygen in a single pass across the culture tank at the maximum cumulative feed burden, all else equal. Assuming a respiratory quotient of 1 kg (range 0.85 to 1.4 kg) carbon dioxide is produced for every 1 kg of dissolved oxygen consumed, this would produce approximately 8.8-12 mg/L of carbon dioxide in a single pass across the land-based culture tanks at the maximum cumulative feed burden. This suggests that recent tanks have been designed to operate at a lower metabolic loading per unit of flow, which would provide improved water quality throughout the culture tank. This trend to operate at a lower cumulative feed burden and metabolic loading rate per unit of culture tank flow is counter to practices from only a decade earlier and is now possible due to the increased use

of RAS technology. Norway's increased use of RAS has likely come about as a consequence of developments of the technology itself, and due to an awareness in Norway during mid 2000's that natural water bodies could not sustain future increases in smolt production, without increased water reuse.

The use of dual drains can influence tank hydrodynamics, either positively or negatively, with the (1) added flexibility to shift the amount of water withdrawn at different tank locations and (2) inclusion of large structures that are associated with these drains that in turn increase drag and/or displace vortices in the rotating flow.

Survey results will suggest tank dimensions and operating conditions that should be modelled in Part 3 using CFD. CFD benchmarking efforts have been completed, where a circular culture tank of 0.353m³ size with a retention time of 35 min was computationally analysed (Figures 5.9 and 5.10). These numerical

findings were in good comparison with the experimental data by Oca and Masalo (2007).

Conclusions

These survey results are being used to choose facilities to visit in 2016, during Part 2 of the HYDRO project. The empirical data from Part 2 site visits will suggest whether the rotational velocities and oxygen mixing are adequate across the culture tank, and whether inlet or outlet conditions should be adjusted. In 2016, work at Nofima will begin to develop computational fluid dynamic models (Part 3) that can suggest how to control water rotational velocities and mixing within large circular tanks.

References

Oca, J., Masalo, I. 2013. Flow pattern in aquaculture circular tanks: Influence of flow rate, water depth, and water inlet & outlet features. *Aquacultural Engineering* 52, 65-72.

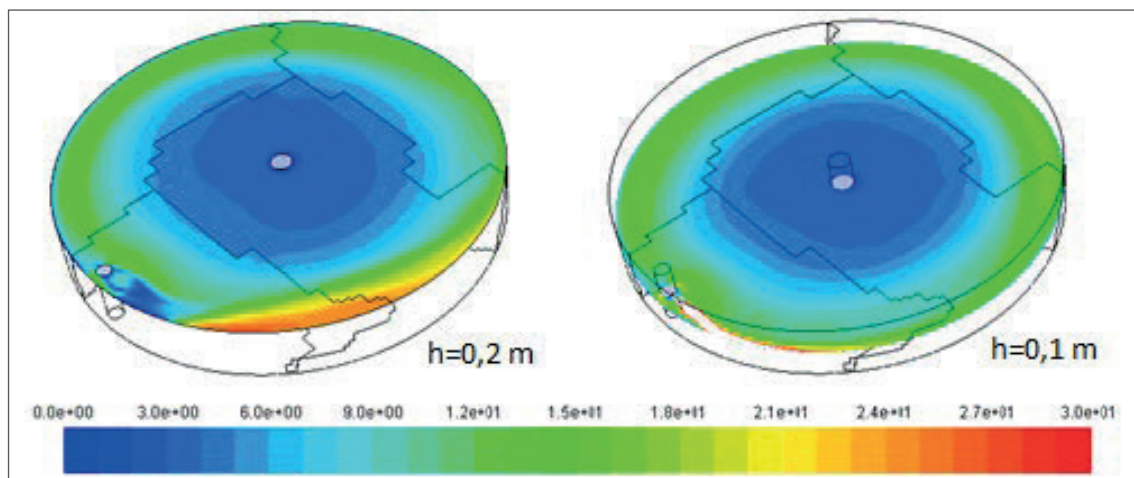


Figure 5.9. Contours of velocity magnitude in cm/s on the free surface and in the plane of flow inlet

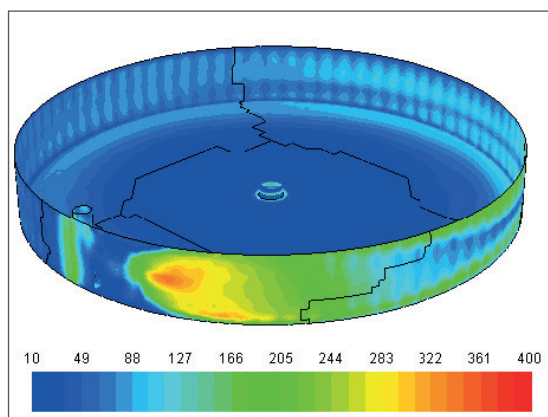


Figure 5.10. Vorticity distribution in s-1 on the tank surface

User specific projects

PARTICLE - Particle tolerance in post-smolts reared in RAS

Project leader: Astrid Buran Holan, Nofima
R&D partners: Bendik Fyhn Terjesen, Jelena Kolarevic, Sven Martin Jørgensen, Sigurd Handeland, Marco Vindas, Tom Ole Nilsen, Lars Ebbesson, Steve Summerfelt, Chris Good
User partners: Krüger Kaldnes, Grieg Seafood, Bremnes Seashore

Background

Recirculating aquaculture systems (RAS) generate small-sized particles; usually below 60 µm in size, the normal cut-off in mechanical filters. The particles originate from biofilm shedding, biofloc (dead and living bacteria), feed and feces. The smallest-sized particles are termed microfines (particles < 30 µm) and colloids (< 1 µm). Moving bed bioreactors (MBBR), commonly used in RAS for salmon smolt production are often claimed to result in higher levels of small-sized particles compared to fixed bed reactors. This is due to the constant abrasion between bioreactor media chips in a MBBR. High levels of suspended solids will increase heterotrophic bacterial growth, which in turn can reduce total ammonia nitrogen (TAN) removal efficiency and deteriorate water quality. Hence, in terms of water treatment efficiency, particles must be tightly controlled. However, although it has been stated that suspended solids in RAS should be kept below 15 mg/l (Thorarensen and Farrell, 2011), experimental evidence on adverse effects of microfines and colloids on salmon post-smolt welfare, health and performance is lacking. Therefore, it is not clear to what extent the smallest-sized particle fractions in RAS must be removed.

Much knowledge exists on effects of solids on fish in their natural habitat, showing adverse effects on stress level, growth, disease resistance, and gill tissues (e.g. Bash et al., 2002;

Chapman et al., 1987). In contrast, knowledge is scarce regarding effects of low concentrations of solids in a culture environment. As pointed out by John Colt "The greatest water quality uncertainty in high intensity reuse systems is the potential impacts of fine solids and organic compounds." (Colt, 2006).

Hence, the tolerance that post-smolts have towards particles is critical knowledge. If there is no detectable need to keep microfines and colloids below a certain concentration and/or size range, this can reduce production cost in closed-containment aquaculture systems.

Materials and Methods

This project is the first part of the PARTICLE project. In this part of the project, the fo-



Figure 5.11. Particle removal equipment at an Atlantic salmon smolt production site. Photo: Bendik Fyhn Terjesen

cus was to establish a reliable experimental system, to prepare an experimental plan and to start the trial at the end of the year. In the second part of the PARTICLE project the trial will be conducted and the adverse effects of microfines and colloids on salmon post-smolt welfare, health and performance will be investigated.

The **first** task was to determine the typical ranges of total suspended solids (TSS) concentrations at user partners. The mean particle concentration was determined in large-scale RAS for postsmolts at Lebesby, Grieg Seafood, and in a brood-stock semi-commercial scale RAS at Nofima Center for Recirculation (NCRA) at Sunndalsøra. Concentrations well above and below this will be included in the experiment.

The **second** task was to establish a reliable system for particle dosing. Particles in RAS exhibit a wide range in stability, size, composition, and shape, and achieving a stable experimental system will be a challenge. An experimental system was designed in which particles were delivered from an overloaded RAS at Nofima, i.e. in which particle concentration are above c. 25 mg/l, by bypassing the mechanical filter and tank particle trap.

A system with five different dilutions of water from the overloaded RAS was fed into 0.5 m³ tanks. The dilution water could not be different from the water carrying the particles, otherwise the particles would not be the only experimental factor. A membrane with cut-off in the nm range was therefore used to filtrate water from the same overloaded RAS, to be used in dilution. The membrane would not change the water quality except for remove microfines. TSS, particle size distribution (PSD), turbidity and other water quality variables was studied over several days to ascertain treatment stability. If this set-up did not perform satisfactorily, another approach would be attempted.

The **third** task was to establish an experimental plan based on task 1 and 2, and to start the experiment.

Results and Discussion

Mean particle concentration was determined by measuring the concentration of TSS in a large-scale RAS for postsmolts at Lebesby, Grieg Seafood (Figure 5.12 A and B), and in a brood-stock semi commercial scale RAS at Nofima Center for Recirculation (NCRA) at Sunndalsøra (Figure 5.12 C). In these system

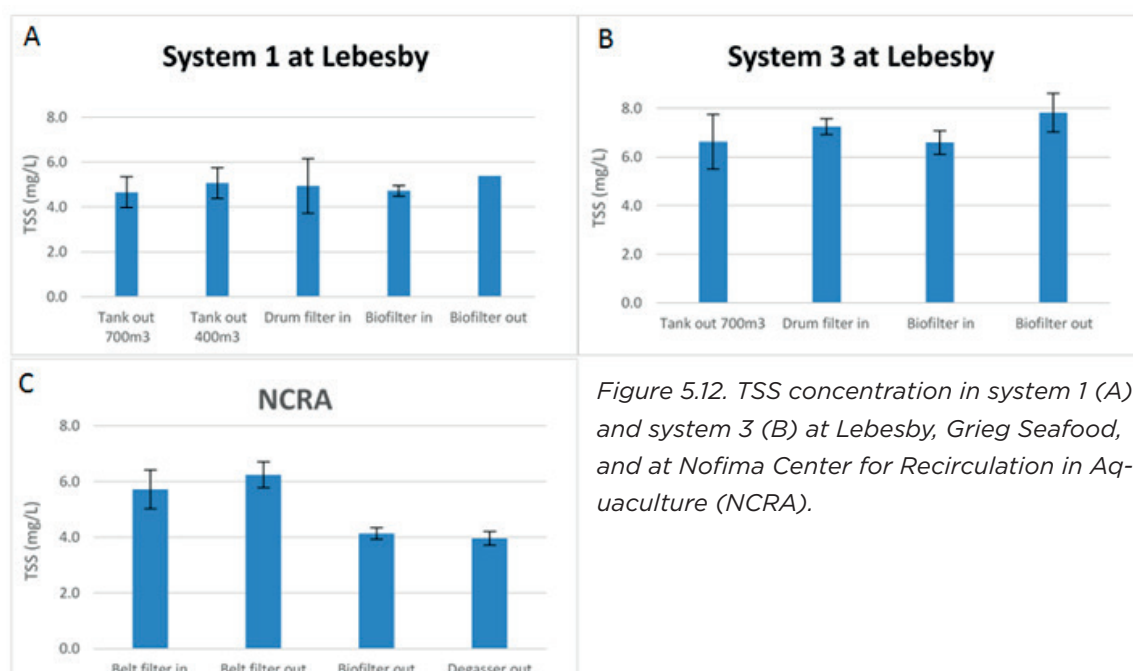


Figure 5.12. TSS concentration in system 1 (A) and system 3 (B) at Lebesby, Grieg Seafood, and at Nofima Center for Recirculation in Aquaculture (NCRA).

the particle concentrations in the water coming out of the fish tanks varied between 5 and 7 mg TSS/L. Concentrations well above and below this will be included in the second part of PARTICLE when designing a test system with five different particle concentrations in the water.

To establish a reliable system for particle dosing, a membrane with a cut-off in the nm range was used to filtrate water from the same overloaded RAS, to be used in dilution. The membrane was a pilot-scale ultrafiltration plant (Aquantis, Veolia) with a cut-off of 20 nm and the capacity of producing 5 m³/h of permeate (clean water). The plant was equipped with an automatically backwashing system to remove fouling and clogging of the membrane. The membrane filtrated water from the overloaded RAS. In the first period of the test the particles in RAS was generated by adding feed into the tanks, and by by-passing the mechanical filter and the tank particle trap. This created a very turbid water with a seemingly high content of fat. The membrane system did not handle this type of water quality well which resulted in severe fouling and filtration arrest. In the last week of the test period, an effort was made to change the type of particles from feed debris to faeces waste by introducing fish in the system. Unfortunately, the membrane did not recover fully after the rough first testing period, and the effort in using the membrane for the experiment was terminated. Since this set-up did not perform satisfactory in creating five different particle dilutions, another approach was attempted.

To create a particle-rich water, faeces from post-smolts stocked in tanks (3.3 m³) in NCRA research hall 1 will be collected from the mechanical filter (beltfilter, Salsnes), concentrated to a particle concentration of 50 mg/L and diluted with brackish water (12 ppt) to give five different particle dilutions. Post-smolt will be stocked in five tanks (0.5m³) in triplicate receiving the five different dilutions

of particle, in a selected range from 0 to 50 mg TSS/L.

An experimental plan is prepared and part 2 of PARTICLE will start in the beginning of March 2016. In the trial the adverse effects of microfines and colloids on salmon post-smolt welfare, health and performance will be investigated.

Summary and Conclusions

Typical ranges of total suspended solids (TSS) concentrations found during the first task ranged between 5 and 7 mg TSS/L. Concentrations well above and below this will be included in the second part of PARTICLE when designing a test system with five different particle concentrations in the water. The selected range is from 0 to 50 mg TSS/L.

The membrane did not handle the poor water quality that was created from dissolved feed, most likely due to the high amount of fat released into the water. The membrane would likely perform better in a conventional RAS, however, for this trial an alternative approach was attempted. To create particle rich water, particles from a recirculating system stocked with post-smolts is collected from the mechanical filter and diluted with brackish water (12 ppt. S) giving five different particle concentrations. The different particle dilutions are led to five tanks (0.5 m³) in triplicate per treatment, stocked with post-smolts. The experiment will have a starting density of 8 kg/m³.

References

Bash, J., Berman, C., & Bolton, S. 2002. *Effects of Turbidity and Suspended Solids on Salmonids* (pp. 92): Washington State Transportation Center (TRAC).

Chapman, P., Popham, J. D., Griffin, J., Leslie, D., & Michaelson, J. 1987. *Differentiation of physical from chemical toxicity in solid waste fish bioassays. Water, Air, and Soil Pollution,*

33(3-4), 295-308. doi: 10.1007/BF00294198

Chen, S., Ling, J., & Blancheton, J.P. 2006. Nitrification kinetics of biofilm as affected by water quality factors. *Aquacultural Engineering* 34, 179-197.

Colt, J. 2006. Water quality requirements for reuse systems. *Aquacultural Engineering*, 34(3), 143-156.

Thorarensen, H., & Farrell, A. 2011. The biological requirements for post-smolt Atlantic salmon in closed-containment systems. *Aquaculture* 312, 1-14.

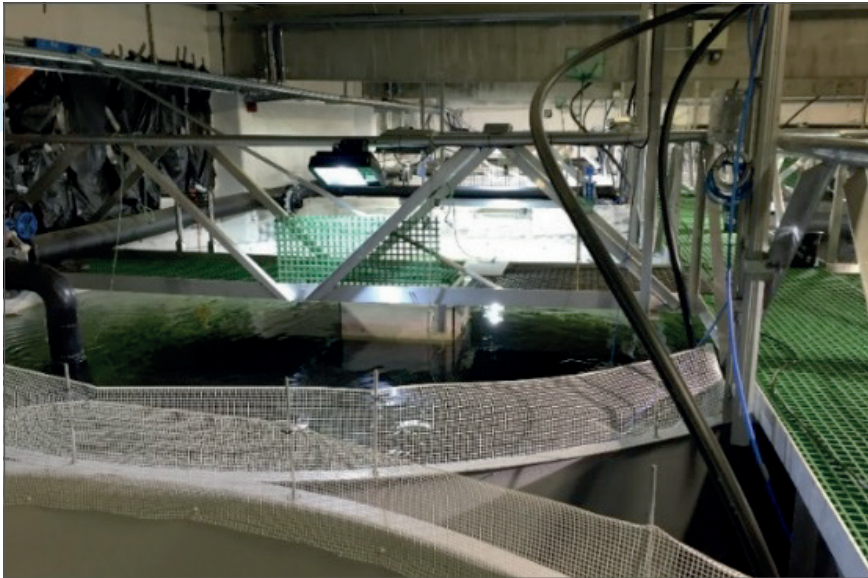


Photo: Grieg Seafood, Lebesby.
Yuriy Marchenco

BIOMASS - Machine vision for biomass in closed systems

Project leader: Jelena Kolarevic, Nofima

R&D partners: Åsa Espmark, Bendik Fyhn Terjesen

User partner: Storvik Aqua AS

Background

The increase in the size of the rearing units on land and in the sea and the increased number of fish in each unit makes it important to develop efficient technology for fish and environment monitoring in aquaculture systems. Currently the biomass is estimated by use of biomass frames that require fish to swim through which has often proved to be challenging. Recent development in camera technology and its combination with the automatic image processing enable new applications that could be useful for non-invasive

measurement of the biomass that would not require period of adaptation for the farmed fish.

Storvik Aqua AS and EBTech have developed the AkvaVision system for biomass estimation that has been successfully tested in sea cages and is commercially available. AkvaVision is a fully automated, camera-based biomass estimator.

The objective of this project is to further develop and customize the AkvaVision system for use in closed-containment aquaculture systems on land and in floating semi-closed containment systems in the sea for Atlantic salmon post-smolt production.

Materials and methods

For the purpose of testing the AkvaVision system in RAS, different system designs and

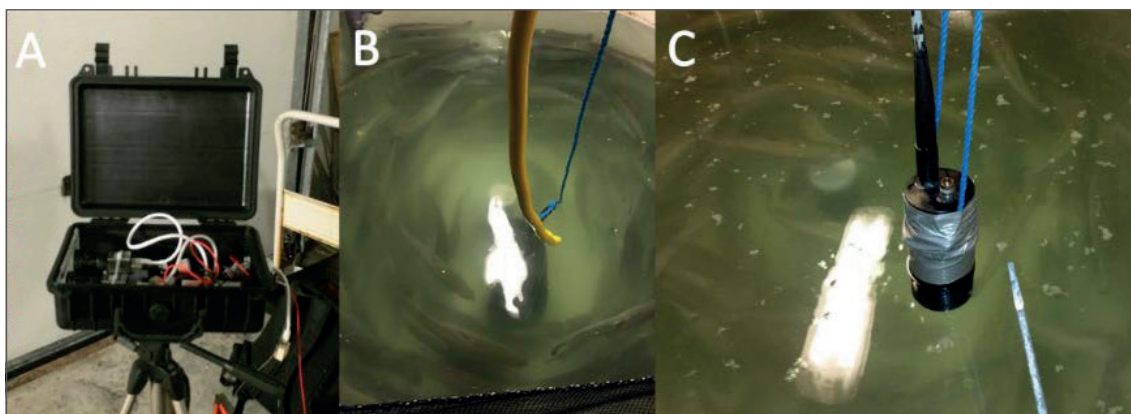


Fig. 5.13 Different designs and locations of the AkvaVision system tested in RAS (description in the text)

system placement were tested out (Fig 5.13): mounted in a suitcase located outside of the tank (A), and built into an underwater housing submerged in water (B) or at the water surface directed downwards (C).

The testing of the AkvaVision system was done at the Nofima Center for Recirculation in Aquaculture (NCRA) in Sunndalsøra. The octagonal 3.3 m³ tanks were provided with brackish 12 ppt water that was treated mechanically and biologically, and was stripped of carbon dioxide and oxygenated on the way back to the tanks (>85% oxygen saturation). A wooden frame was made and placed around the window on the inner side of the tank when the system was located outside the tank (Fig 5.14B). One of the tasks of the project was to find the optimal positioning of the system that would ensure that high quality images are taken for further analysis and estimation of the biomass. In total three different position of the system in regard to the rearing tank were tested: 1) outside the tank facing the plexiglas window and directed inwards against the center of the tank (Fig 5.14A); 2) submerged in the center of the rearing tank (Fig 5.13B); 3) Just below the water surface level (Fig 5.13C). Another task of the BIOMASS project in 2015 was to determine the effect of different post-smolt density on the accuracy of the biomass estimation using the AkvaVision system in RAS. The accuracy of the system was tested using

post-smolts of the average individual weight of 870 g at two different densities, 50 and 10 kg/m³. The post-smolts were bulk weighed and stocked in two 3.3 m³ tanks at density of 50 kg/m³ at the start and the bulk weighing was repeated three times during after which the density in the tanks was reduced to 10



Fig 5.14. The two 3.3 m³ tanks at Nofima Center for Recirculation in Aquaculture with plexiglas windows used for the testing of the AkvaVision system in RAS (A). The wooden frame was added to the tanks when the AkvaVision system was placed outside the tank (C).

kg/m³ and fish were bulk weighed on two occasions. The fish were exposed to 24h light regime in order to ensure that the estimation of biomass was possible during the entire 24h. Experimental tanks were filmed for approximately 30 minutes using Go Pro camera placed above the rearing tanks in order to analyse the position of the fish in the tank and in the relation to the camera system.

Results and discussion

During the testing of different AkvaVision designs in RAS the presence of particles was challenging for the image analysis process as the particles created noise in the analysed images. This was in particular observed in situations when the penetration of the available light was limited, such as during periods with higher density in the tanks and when the camera was submerged in the middle of the tank.

Water from RAS is also biologically active with large number and amount of microorganisms present in all compartments of the system. Biofouling is often observed on the surface of the equipment or different type of in-line probes, as described by Kolarevic et al. (2011) which can reduce the accuracy of the equipment that is in constant contact with the RAS water. In order to avoid some of the above mentioned issues and to insure minimum maintenance for the AkvaVision system, we have tested the system accuracy while it was placed outside the tank facing inwards through a tank window. In the situation when the density in the rearing tank was about 50 kg/m³ and above fish were evenly distributed along the tank edges and very close to the tank window. The vicinity of the fish to the camera system prevented the system from taking the necessary images of the whole fish that would allow for the biomass estimation. The attempts to create distance between the camera and the fish in the tank did not lead to the increase in number of approved images at the same density, but had a positive effect

when the density in the system was reduced to 10 kg/m³. In this case only one or couple of fish were in the visibility field of the cameras and together with the improved light condition in the tank could have contributed to the biomass estimation done by AkvaVision system.

The visibility field was larger when the system was submerged in the middle of the tank and the presence of the system in the tanks did not have any visible effect on the fish behaviour. And although it was possible to take good raw images of the fish calibration of the system and the lack of light caused by high density and turbidity level (over 3 NTU) prevented any further analysis.

Use of the camera system just below water surface was challenging due to the backscattering and absorption of visible light caused by suspended particles and organic matter in the water. Certain intensity of visible light will always be present at the water surface making this approach less feasible to pursue.

The results from this study indicate that the estimation of the biomass in at higher rearing densities (50 kg/m³) in the RAS was challenging for the tested systems due to the low light

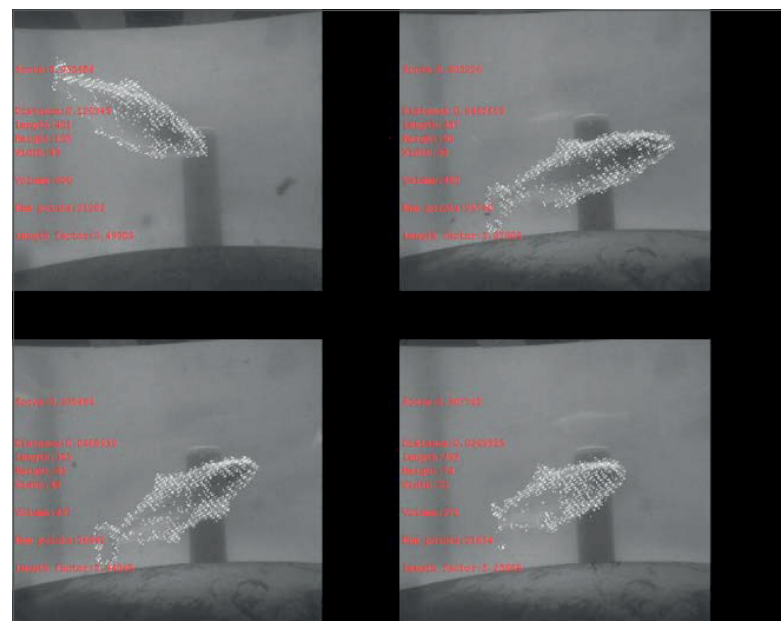


Fig 5.15. The result images of the Atlantic salmon post-smolt used for estimation of the average weight and biomass

intensity, water turbidity and the close vicinity of the fish as described above. The AkvaVision system was able to perform necessary calculations and estimate the average individual weights in the tank when density in the tanks was reduced to 10 kg/m³ (Fig 5.15). At the lower rearing densities in the rearing tanks the AkvaVision system underestimated the biomass in the tanks which was partially related to the small number of the system approved images that were used for the biomass calculation. The largest number of daily approved images during this period was 2-7 times lower than when the system is used in the sea cages. In addition the estimation of the biomass was done mainly for one of the two replicate tanks in the experiment. The behavioural analysis indicated that the lack of the reproducibility of the biomass estimation in the tanks was due to the different location of the fish in the tanks in relation to the camera system location (outside the tank facing the window in one of the tank walls) and the level of their activity. In the tanks where larger number of images were obtained fish were more active and were mainly standing in the visibility field of the camera.

In the other tank fish were not as active and were mainly located close to the tank wall outside the camera visibility field. This was also confirmed by the manual analysis of the images from the two tanks taken on one of the experimental days. Turbidity during the experimental period with higher rearing density was around 3 times higher than when the fish density was 5 times lower in the tanks. A more systematic effect of turbidity on the efficiency of the AkvaVision system will be further tested.

In conclusion, the results of the testing reported in this deliverable indicate that the AkvaVision most optimal location would be fully submerged in the tank with the ability to cover different parts of the tank that would be particularly important at the low stocking densities. The large number of daily approved

images will be a prerequisite for the AkvaVision system ability to estimate the biomass accurately.

There are indications that both higher and lower densities in the tank can provide different challenges when the biomass estimation is concerned. Therefore, the effect of the rearing densities on AkvaVision system biomass estimation in RAS will be evaluated again using the optimal design of the system. The results of the testing period also indicated that the largest challenges for the optimal operation of the system were the turbidity of the water and the lack of optimal light in the tanks. Further testing of the system in CtrlAQUA will address those issues.

References

Jonsson B., Jonsson N. 2011. *Ecology of Atlantic Salmon and Brown Trout: Habitat as a template for life histories*. Springer Dordrecht Heidelberg London New York, p.707.

Kolarevic, J., Ciric, M., Zühlke, A., Terjesen, B.F., 2011. On-Line pH Measurements in Recirculating Aquaculture systems (RAS), *Aquaculture Europe (2011)*. European Aquaculture Society, Greece, Rhodes, pp. 570-571.

Terjesen, BF, Summerfelt, ST, Nerland, S, Ulgenes Y, Fjæra SO, Megård Reiten BK, Selset R, Kolarevic J, Brunsvik P, Bæverfjord G, Takle H, Kittelsen A, Åsgård T 2013. Design, dimensioning, and performance of a research facility for studies on the requirements of fish in RAS environments. *Aquaculture Engineering* 54, 49-63.

REMOVAL - Pre-project, new technologies for RAS sludge processing

Project leder: Turid Synnøve Aas, Nofima

R&D partner: Bendik Fyhn Terjesen

User partner: Storvik Aqua AS

Background

Aquacultural sludge contains considerable amounts of energy and nutrients, there among phosphorus. In this project, a new technology for reclamation of phosphorus from sludge was tested.

The sludge from aquaculture contains large amounts of unutilized nutrients and energy. The production in land-based and semi-closed systems in Norwegian aquaculture is increasing, resulting in an increased production of aquacultural sludge from such farming systems. Phosphorus (P), which is a limited resource, has low and variable digestibility from salmon feed. Assuming 35% digestibility, 65% of the P in salmon feed is excreted in the faeces. Salmon feed contains roughly 1% P, and the P lost in faeces thus sums up to considerable amounts. Likewise, salmon is fed high-energy feeds where approximately 20% of the energy is transferred to faeces. In addition, the sludge contains variable amounts of feed spill. Clearly, capturing and utilizing

particles from sludge is necessary for effective utilization of the feed resources.

Hias IKS (Hamar, Norway) has developed technology for a biological method for biological P removal from the water fraction of waste water from sewerage. The method allows cellular bacterial incorporation of phosphate, which is the dissolved fraction of P. Dissolved P is removed from water by means of bacteria which are capable of absorbing up to 30 % of their biomass as P. In this project, the possibility of reclamation of P from aquacultural sludge with Hias' method was tested.

Furthermore, the possibility of using the Cambi process for biogas production from sludge was briefly investigated. However, this technology seemed to depend on large, central plants to be economically sustainable, and was therefore not further examined.

Materials and methods

The amount of dissolved and total P was analysed in samples collected at Nofima's RAS facilities (NCRA). The following samples were collected: 1) sludge from one of the swirl separators that are fitted to each tank (primary filter), 2) sludge from the Salsnes filter (secondary filter) and 3) water which enter the biological filter, after Salsnes filter (Fig. 5.16). At the current time, only 12 % brackish

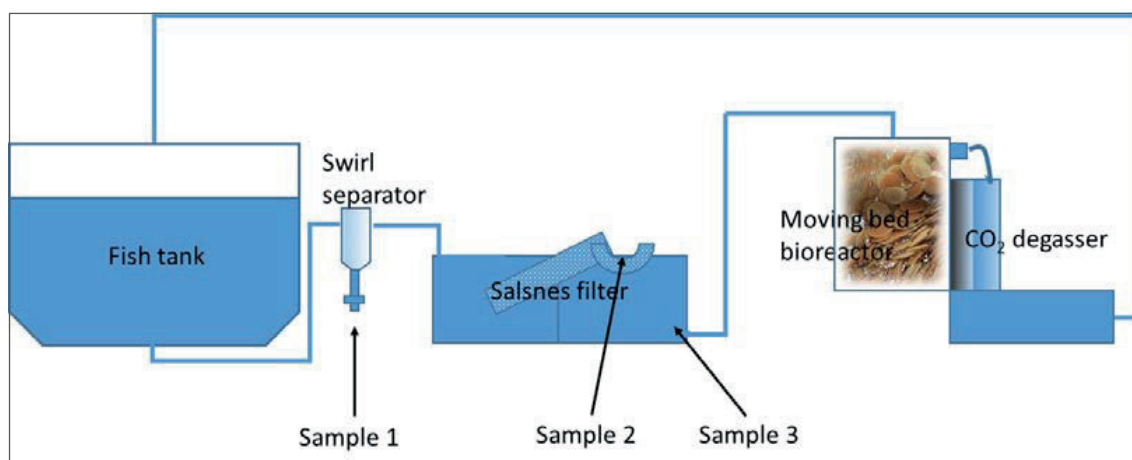


Figure 5.16 A simple overview of the RAS. Samples were collected at 1) the outlet of the swirl separator, 2) at the Salsnes filter, and 3) the water which enter the biological filter.

water was used in the system and consequently, the collected samples was from 12 ‰ brackish water.

The samples were frozen and stored at -20 °C, and thawed over night before analysis.

The amount of total and dissolved P was measured in the three samples (Table 5.2). Dissolved P was measured photometrically as PO₄ after filtering at 1.2 µm and reaction with sulphuric acid and a colouring agent. The biological method for reclamation of PO₄ was tested on a solution prepared from 5 % of water filtered from sludge from the Salsnes filter (sample 2) and 95 % of water before biofilter (sample 3). Taking representative samples of sludge is challenging, particularly at the Salsnes filter. Furthermore, prior to analysis, dilution of the sludge samples was required. Thus, the results represent approximate, and not absolute, values.

Results

The biological method for reclamation of PO₄ was tested on a solution prepared from 5 % of water filtered from sludge from the Salsnes filter (sample 2) and 95 % of water before biofilter (sample 3). Within six hours,

98.5 of the PO₄ was reclaimed. Also, NH₄⁺ was reduced by 75 %. Thus, Hias' method for reclamation of PO₄ seemed to function in the water fraction of aquacultural sludge with 12 ‰ brackish water.

The highest concentration of P was found in sludge from the swirl separator (Table 5.2). Only 10%, or 506 mg/L water soluble PO₄ (corresponding to 167 mg P/L) out of 1700 mg total P were found in the sludge from the swirl.

Discussion

Although developed for fresh water, Hias' method functioned well for reclaiming dissolved P from aquacultural sludge from 12‰ brackish water in this simple test. However, only 10% of the P from the sludge was found in the water soluble fraction. In total, this technology therefore has limited effectivity for reclaiming P from aquacultural sludge with today's feeds.

Conclusion

The highest concentration of P was found in sludge from the swirl separators. P in the sludge was predominantly bound to particles (90 %).

Table 5.2 Chemical analysis of sludge

Sample	Dissolved PO ₄ (mg PO ₄ /L)	Total P (mg P/L)	NO ₂ (mg NO ₂ -N/L)	NO ₃ (mg NO ₃ -N/L)	NH ₄ (mgNH ₄ -N/L)	sCOD (degradable organic matter, mg/L)
1) Sludge from swirl separator	506	1700			380	
2) Sludge from Salsnes filter	105	1350			120	
3) Water before bio-filter	0.53	0.52	0.02	3	0.5	55

For uptake of dissolved phosphate, Hias' method functioned well on a sample with 12 ‰ brackish water. However, since approximately 90 % of the P was bound to particles, actions that release water dissolved PO_4 from particles are necessary for this method to be efficient for reclamation of P from aquacultural sludge.

MICROPARASITES - Characterization of microparasites in closed and semi-closed containment systems

Project leader: Are Nylund, UIB

R&D partners: Heidrun Plarre, Ida Rud, Sven Martin Jørgensen, Harald Takle, Sigurd Handeland

User partners: Pharmaq, Pharmaq Analytiq, Oslofjord Ressurspark

Background

The production line of Atlantic salmon in Norway is influenced by a range of different microparasites (viruses, bacteria, parasites, fungi) where some of these are transmitted via brood fish while others are horizontally transmitted through fresh or sea water or via vectors (See: Nylund et al., 2015). These microparasites include some that are highly virulent in dense populations (within farms) while others may give sublethal infections

and persist for long periods within farmed populations. Many of these microparasites may become an even larger problem if introduced to closed or semi closed containment systems (CCS) with even higher population densities and a slower exchange of water. In addition, there is a steady emergence of new pathogens in Norwegian salmon farming. A change from open net cages to S-CCS may have a positive influence and lead to a reduction in the emergence of new pathogens, but S-CCS in sea water, using untreated intake water, may result in the emergence of pathogens that otherwise would not have been introduced to farmed Atlantic salmon. If oxygen levels drop or if biofouling becomes a problem in CCS one may even expect increased problems with pathogens that in most cases give sublethal infections only. Preline (S-CCS) included in this study has a fast water exchange even compared to open cages. The main objective of this preliminary study is to identify the most important known microparasites in a S-CCS (Preline) located in western Norway, close to Bergen. The major focuses are on differences in diversity, prevalence and load of microparasites in the S-CCS compared to nearby open cages with salmon of the same origin.

Materials and Methods

The salmon smolt at two production sites, Preline (S-CCS) and site Rongøy (open cage B), came from the same population at the

Table 5.3. The salmon in Preline (S-CCS) were moved to an open cage (A) in the beginning of September. The salmon in the open cage B were kept at the same location throughout the study period.

Site	Start date	Number	Weight	Lice/AGD treatment	Lice average
Preline	19.05.2015	157501	124 gr	none	0**
Open cage A	1-9.09.2015	153280	548 gr	none	0,22**
Rongøy					
Open cage B	8.04-1.05.2015	193600	123 gr	3* (23.09, 6.10, 29.10)	0,18**

*Treatments against AGD. **Average number of mobile stages and mature females.

same smolt production site. The marine production were kept at two separate locations in Hordaland County where both areas are known to harbor the same large variety of microparasites in addition to salmon lice (*Lepeophtheirus salmonis* and *Caligus elongatus*) (Table 5.3). Salmon produced in Preline were moved to a new location in Hordaland in the beginning of September (Table 5.3).

Atlantic salmon (N = 30 at each sampling) were collected at the sites after some month's production in sea water; Preline sampled the 27th August, Cage A the 14th of October, and Cage B the 26th of August and the 8th of October. The salmon were tested for presence of 13 different microparasites using real time RT PCR: Infectious salmon anemia virus ISAV (Plarre et al., 2005), salmonid gill pox virus (SGPV, Nylund et al unpublished), Salmonid alphavirus (SAV, see Andersen et al 2007), Piscine orthoreovirus (PRV, Repstad, 2011), Piscine myocarditis virus (PMCV, unpublished), Infectious pancreatic necrosis virus (IPNV, Nylund et al 2011), *Ca. B. cysticola* (Tolås, 2012), *P. theridion* (Nylund et al., 2010), *Ichthyobodo salmonis* (Isaksen et al 2012), *Paramoeba* spp (Nylund et al unpublished), *Tetramitus* sp (Nylund et al unpublished), *P. pseudobranchicola* (Nylund et al 2011) in addition to a standard gene (elongation factor alpha) (See Andersen et al 2007). *Tenacibaculum* spp. were cultured from the salmon (see Småge et al., 2015).

A manual extraction of RNA, using the ISOL protocol, has been performed for all samples included in this study. This method has been chosen since it gives a superior quality of the RNA compared robot-extractions.

S-CCS: Preline

Preline differ from most other S-CCS due to the fact that the water exchange is fast (4 -5 minutes), even compared to open cages, while the water exchange in other S-CCS may be as high as 1 hour. The water intake is locat-

ed between 30 and 40 meters.

Definitions

The diversity index (DI) is defined as the percentage of the total number of different microparasite species present in the tested population (given as a range from 0 - 10). The prevalence of a specific microparasite is the percentage of positive individuals in the population tested.

The mean load of a specific microparasite is calculated 50 minus the mean ct value ($ML = 50 - Ct^t$) for the positive individuals in the tested population (Possible range: 12 - 45 where 45 is the highest possible load).

Results

A major focus of this project was on monitoring the diversity, prevalence and load (density) of microparasites in Preline (S-CCS) compared to production in an open cage, cage B. Smolts from the same population were stocked at both sites (both stocked in May 2015). The fish were sampled in August and October and tested for presence of 13 different microparasites (real time RT PCR: ISAV, SGPV, SAV, PRV, PMCV, IPNV, *Ca. B. cysticola*, *P. theridion*, *Ichthyobodo salmonis*, *Paramoeba* spp, *Tetramitus* sp, *P. pseudobranchicola*, Culture: *Tenacibaculum* spp.) in addition to a standard gene (elongation factor alpha). The diversity of microparasites, at the first sampling (august 2015), was higher in the open cage (9 different microparasites, DI = 6.9) compared to Preline (6 different microparasites (including cultures of *Tenacibaculum* spp), DI = 4.6). The prevalence of *Ca. B. cysticola*, PRV, IPNV and *P. theridion* were the same at both sites, while the prevalence of *P. pseudobranchicola* was much higher in the open cage, ie. 3.3 % vs 80% (Figure 5.17). The load (density) of *Ca. B. cysticola* and *P. theridion* were significantly higher in the open cage compared to Preline (S-CCS). The load of PRV, IPNV and *P. pseudobranchicola* were

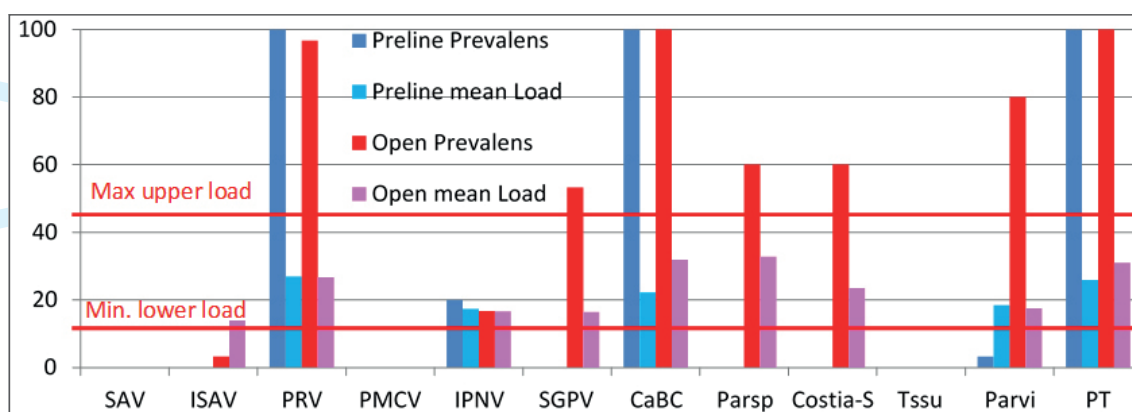


Figure 5.17. Prevalence (%) and load (density) of microparasites on the gills of salmon in open cage A (blue) versus open cage B (red). The maximum (45) and minimum (12) loads are marked with red lines in the figure. The salmon in cage A were moved from Preline to this cage 45 days before sampling, while the salmon in cage B were kept in this open cage throughout the sampling period.

low at both sites (carrier status). The load of *Tenacibaculum* spp in Preline was not calculated, but the salmon showed a few sign of tenacibaculosis.

The mortality in the cage B was slightly higher compared to the mortality in Preline. None of the populations were treated against salmon lice but the population in the cage B was treated twice against AGD (The first treatment was unsuccessful).

The salmon from Preline was moved to an

open cage (cage A) in the beginning of September 2015. New samples were collected from both populations in the middle of October, ie. 1 ½ month after the fish had been moved from Preline to cage A. The samples from October were tested for 12 different microparasites (real time RT PCR: ISAV, SGPV, SAV, PRV, PMCV, IPNV, Ca. B. cysticola, *P. thetidion*, *Ichthyobodo salmonis*, *Paramoeba* spp, *Tetramitus* sp, *P. pseudobranchicola*.). The diversity in cage B showed presence of seven different microparasites (DI = 5.8) while the diversity in cage A showed presence of six

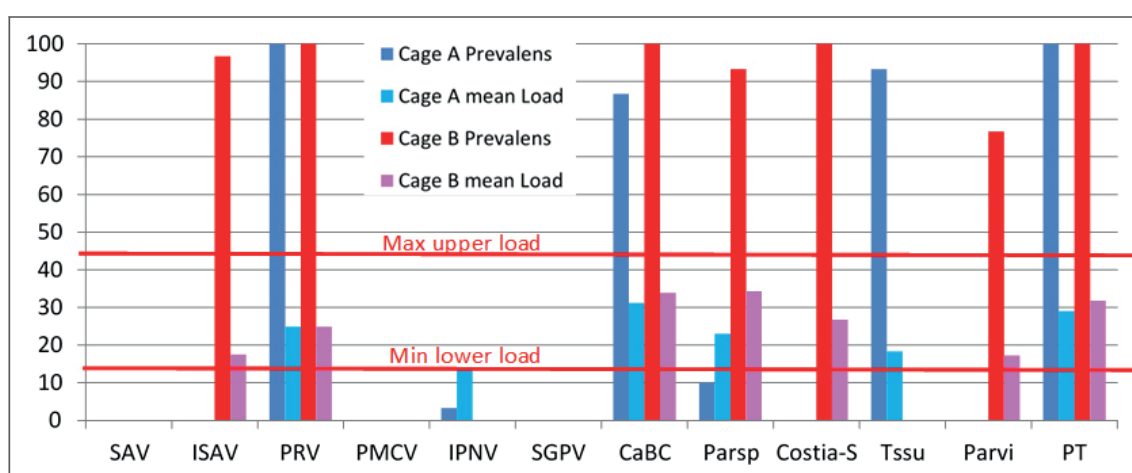


Figure 5.18. Prevalence (%) and load (density) of microparasites on the gills of salmon in open cage A (blue) versus open cage B (red). The maximum (45) and minimum (12) loads are marked with red lines in the figure. The salmon in cage A were moved from Preline to this cage 45 days before sampling, while the salmon in cage B were kept in this open cage throughout the sampling period.

microparasites (DI = 5.0). The prevalence of *P. theridion* and PRV was 100 % in both cage A and B, while the prevalence of *Ca. B. cysticola*, and *Paramoeba* spp. was higher in cage B (Figure 5.18). The prevalence of the amoeba *Tertramitus* sp. was 93.3 % in cage A while the fish in cage B were negative. *I. salmonis* and ISAV were not present in cage A while the prevalence was close to 100 % in cage B. The prevalence of *P. pseudobranchicola* was 76.7 % in cage B (not present in cage A). The load (density) of *Ca. B. cysticola*, *P. theridion* and *Paramoeba* sp. were higher in cage B compared to cage A, while the density of PRV was the same at both locations.

Discussion

These preliminary results suggest that S-CCS (Preline) may prevent the exposure of salmon from some of the microparasites included in this study. However, the sample size is very limited with respect to number of cages (S-CCS/Open), and Preline and the open cage (B, Rongøy) were not kept at the same location. Still, the salmon were of the same origin (same smolt production site) and some of these microparasites (SAV, ISAV, PRV, *Ca. B. cysticola*, *Paramoeba* spp, *Ichthyobodo salmonis*, and *P. theridion*) are known to be quite ubiquitous in the study area. The microparasites, ISAV, IPNV, PRV, and *Ca. B. cysticola*, are also frequently found in smolt production sites and may have followed the smolt from fresh water and into Preline and cage B. Screening of the smolt from the fresh water site will show if these microparasites followed the fish into the sea. The only microparasites present in Preline that must have been acquired in the sea are *P. pseudobranchicola* and *P. theridion*. These two parasites have a complex life cycle involving other hosts in sea water. The ISA viruses detected are of the low virulent type, termed HPRO.

Conclusion

The diversity index is lower in Preline com-

pared to the open cage (cage B), while the prevalence and load (density) are equal or lower in Preline compared to cage B. Hence, production of salmon in Preline (S-CCS) do not seem to have a negative effect on the prevalence and loads of microparasites compared to open cages.

References

- Andersen, L., Bratland, A., Hodneland, K., Nylund, A. 2007. Tissue tropism of salmonid alphaviruses (subtypes SAV1 and SAV3) in experimentally challenged Atlantic salmon (*Salmo salar* L.). *Arch Virol.* 2007;152, 1871-83. Epub 2007 Jun 20.
- Apablaza, P., Løland, A.D., Brevik, Ø.J., Ilard, P., Battaglia, J., Nylund, A. 2012. Genetic variation among *Flavobacterium psychrophilum* isolates from wild and farmed salmonids in Norway and Chile. *J Appl Microbiol* doi: 10.1111/jam.12121
- Apablaza, P., Brevik, Ø.B., Mjøs, S., Valdebenito, S., Ilardi, P., Battaglia, J., Dalsgaard, I., Nylund, A. 2015. Variable Number of Tandem Repeats (VNTR) analysis of *Flavobacterium psychrophilum* from salmonids in Chile and Norway. *BMC Veterinary Research* 11:5150 (DOI 10.1186/s12917-015-0469-7).
- Isaksen, T.E., Karlsbakk, E., Repstad, O., Nylund, A. 2012. Molecular tools for the detection and identification of *Ichthyobodo* spp. (Kinetoplastida), important fish parasites. *Parasitology International* 61, 675 – 683.
- Nylund, A., Karlsen, C.R., Good, C., Jørgensen, S.M., Plarre, H., Isaksen, T.E., Handeland, S.O., Wollseth, K., Ottem, K.F. 2015. Review of microparasites that could represent a future problem for production of salmonids in closed or semi-closed containment systems. *CtrlAqua Report* pp 178.
- Nylund, S., Nylund, A., Watanabe, K., Arnesen, C.E., Karlsbakk, E. 2010. *Paranucleospora*

theridion n.gen., n.sp. (Microsporidia, Entero-cytozoonidae) with a life cycle in the salmon louse (*Lepeophtheirus salmonis*, Copepoda) and Atlantic salmon (*Salmo salar*). *J Euk Micr* 57, 95-114.

Nylund, S. Andersen, L., Saevareid, I., Plarre, H., Watanabe, K., Arnesen, C.E., Karlsbakk, E., Nylund, A. 2011 Diseases of farmed Atlantic salmon *Salmo salar* associated with infections by the microsporidian *Paranucleospora theridion*. *Dis Aquat Organ*. 94, 41-57.

Plarre, H., Devold, M., Fridell, F., Nylund, A. 2005. Prevalence of infectious salmon anaemia virus (ISAV) in wild salmonids collected in Western Norway. *Dis Aquat Org*. 66, 71 – 79.

Repstad, O. 2011. Kartlegging av patogendynamikken hos oppdrettslaks (*Salmo salar*) med diagnosen pankreassykdom (PD). Master thesis, Department of Biology, University of Bergen.

Småge, S.B., Brevik, Ø.J., Duesund, H., Ottem, K.F., Watanabe, K., Nylund, A. 2015. *Tenacibaculum finnmarkense* sp. nov., a fish pathogenic bacterium of the family Flavobacteriaceae isolated from Atlantic salmon. *Antonie van Leeuwenhoek* DOI 10.1007/s10482-015-0630-0.

Tolås, I.V. 2012. Lukket merdsystem - AquaDomen: Effekt på smittedynamikk. Master thesis, Department of Biology, University of Bergen.

Økland, A., Nylund, A., Øvergård, A.C., Blindheim, S., Watanabe, K., Grotmol, S., Arnesen, C.E., Plarre, H. 2014. Genomic characterization and phylogenetic position of two new species in *Rhabdoviridae* infecting the parasitic copepod, salmon louse (*Lepeophtheirus salmonis*). *PlosOne* 9(11):e112517.doi:10.1371/journal.pone.0112517.

INTAKE - Particle and pathogen removal from intake water in semi-closed systems

Project leader: Astrid Buran Holan, Nofima

R&D partners: Bendik Fyhn Terjesen, Brian Vinci, Sigurd Handeland

User partners: Cermaq, Aquafarm Equipment

Background

Closed-containment aquaculture systems in sea have the possibility to gain substantial control over the environment inside the culture tank if it is possible to efficiently prevent pathogenic microorganisms from entering via the intake water, thus eliminating infestations of sea-lice and pathogenic microorganisms (e.g. *Moritella viscosa* or *Tenacibaculum*). Treating the intake water could lead to better control of the water quality, reduce mortality and improve fish health and fish welfare. There is a clear need to evaluate if a treatment technology can be and/or should be adapted to such systems. Moreover, the treatment needs to manage the huge intake flows in such systems, without large head loss.

Material and Methods

In this project, the microbial concerns for S-CCS were discussed and a review of available water treatment technologies from other industry areas that can be implemented in S-CCS in sea was performed. Furthermore, the challenges associated with the different water treatment technologies, the operational experiences such as the potential risk in forming harmful by-products, and the energy consumption and investment costs was evaluated. This review is part 1 of the INTAKE project. In part 2 of the project the main goal will be to do a small-scale efficiency test of the best solution from part 1, and describe how this treatment can be implemented in a commercial scale semi-closed aquaculture system in sea. Research and development is needed before any treatments can be integrated in

commercial scale facilities. Part 2 will therefore focus on the reliability and feasibility, the up-scaling of the treatment processes, and efficiency in producing clean water.

Results and Discussion

Microbial concerns

Disinfection of intake water sources to aquaculture rearing systems may be viewed as a necessary biosecurity action or simply as a disturbance of the natural microbial balance. Floating S-CCS can be regarded as huge flow-through systems, where the water flowing into the system will contain a natural microbial community. It may be hypothesized that when exposing the microbial communities in the untreated intake water to a completely new environment with shifting and unstable organic loading, and different nutrient profile and environmental conditions inside the tank, will induce disturbance of the natural microbial balance and growth of opportunistic and potential pathogenic microbial species. On the other hand it may be hypothesized that treating the intake water with UV will as well disturb the natural microbial communities in the water giving potentially opportunistic bacterial species the chance of blooming and proliferating in the S-CCS.

Another concern regarding the microbial aspect around S-CCS is the potential of getting upwelling of sediments from the bottom of the sea, induced by the pumping of huge amounts of water. From other studies we know that marine sediments in the sea will harbor pathogenic species (e.g. *Vibrio spp.*) (Blackwell and Oliver, 2008; Shikuma and Hadfield, 2010) and *Moritella viscosa* (Colwell and Morita, 1964; Urakawa et al., 1998). According to Enger et al. (1989) the fish pathogen *Vibrio salmonicida* was detected in sediment samples from diseased farms several months after an outbreak of the disease, and the bacterium was also detected in a sediment sample from a disease-free fish farm. It may also be hypothesized that

sediments could be a reservoir of the amoeba causing AGD.

Furthermore, there will always be periodically occurrence of pathogens in the sea due to e.g. algal blooms, or upwelling in the sea due to storms, leading to e.g. sea lice in deeper water levels.

Available water treatment technology

Water treatment systems applied in other industry areas could potentially be adapted to treat the huge intake flows in S-CCS. Filtration processes will of course be very effective in removing the biggest microparasites. To remove most of the sea lice stages, filters with pore sizes of less than 200 µm would be needed to filter the copepodite stage (0.7-0.8 mm), which is the infectious stage. Filtration could also be effective for the removal of e.g. the amoeba *Paramoeba perurans* (AGD causing agent), however this will require filters with pore sizes less than 20 µm, and even with such small pores, the amoeba could be able to pass due to its flexible characteristics. A combination of filtration and disinfection with e.g. UV would potentially improve the removal of the amoeba. UV-radiation has been accepted as a reliable method for inactivation of algae and bacteria, however a study of ballast water treatment has shown that some algae species and larger zooplankton are UV-resistant, and that a proper filtration to remove larger UV-resistant zooplankton is needed (Liltved et al., 2011).

Ozone and advanced oxidation process (AOP) are both very effective treatments for removing organic material and inactivating microparasites in the water. However, the potential formation of toxic disinfection by products (DBP) in seawater are limiting the use of these technologies and would require thorough investigations before installations in the intake water to S-CCS.

Placement of the various candidate technologies for inlet water treatment in S-CCS is an unresolved issue. One alternative considered

by some suppliers is to mount the system directly on the floating tank, at the intake water, as is already the solution for waste water treatment of holding pens where a pressurized mechanical filters (Bernoulli filter) is used, followed by a medium pressure UV lamp.

Energy consumption

Costs covers both investments costs and running costs. The investment costs will depend on the size of the water flow, and the choice of technology. The running costs are determined by the energy consumption for pumping, filtration and UV lamp, and also by the cleaning of the filtration and disinfection unit. Table 5.4 shows an example of energy requirements (kW) for pumping, filtration and UV treatment of a flow of 100 m³/min by using a pressurized and a non-pressurized filter.

Interview with suppliers

Several equipment suppliers were contacted for interview, and in total four suppliers presented different water treatment technologies that could be adjusted and implemented to treat the intake water to S-CCS in sea.

Summary and Conclusions

Treating the intake water to semi-closed containment systems (S-CCS) in sea could lead to better control of the water quality, reduced mortality and improved fish health and fish welfare. However, there is a clear need to evaluate if a treatment technology can be and/or should be adapted to such systems. A review of available water treatment technologies from other industry areas that could be implemented in S-CCS in sea was performed, and the associated challenges discussed. Different disinfection techniques and treatment methods such as UV radiation, ozone, advanced oxidation process (AOP) and ultrasound was described, and different filtration installations (pressurized and non-pressurized filters) and potential placements of the various candidate technologies for S-CCS evaluated. The calculated energy requirements (kW) for intake water treatment (pumping, filtration and UV) by using a pressurized and a non-pressurized filter was found to be four and three times higher, respectively, compared to having no filtration and UV treatment. An interview with Norwegian technology suppliers showed that there are several commercial available water treatment technologies in other industry areas today that can be adapted to intake water treatment for S-CCS.

Table 5.4. Example of pumping, filtration and UV power requirements (kW) for treating a flow of 100 m³/min with pressurized filter and UV, non-pressure filter and UV, and no filter – no UV system. For this example a head loss of 0.3 bar is selected for the pressure filters and 0.03 bar for the non-pressure filters, and a lifting height of 1.5 m. The energy for the UV is set to 25 mJ/cm², and a low pressure low intensity UV lamp with 65 watts is selected. The head loss over the UV unit is set to 0.01 bar.

System	Pumping power required (kW)	UV power required (kW)	Total power required (kW)	Annual energy (kWh/year)
Pressurized filter system	126.1	51.6	177.7	1 556 764
Non-pressurized filter system	54.6	72.2	126.8	1 110 951
No filter, no UV system	42.8	-	42.8	374 541

References

- Blackwell, K. D., & Oliver, J. D. 2008. The ecology of *Vibrio vulnificus*, *Vibrio cholerae*, and *Vibrio parahaemolyticus* in North Carolina estuaries. *Journal of Microbiology*, 46, 146-153. doi: 10.1007/s12275-007-0216-2
- Colwell, R. R., & Morita, R. Y. 1964. Reisolation + emendation of description of *Vibrio marinus* (Russell) Ford. *Journal of Bacteriology*, 88, 831-.
- Enger, O., Husevag, B., & Goksoyr, J. 1989. Presence of the fish pathogen *Vibrio salmonicida* in fish farm sediments. *Applied and Environmental Microbiology*, 55, 2815-2818.
- Liltved, H., Tobiessen, A., Vogelsang, C., Bomo, A.-M., & Tryland, I. 2011. UV treatment on ballast water - dose requirements and analytical challenges. Paper presented at the Workshops on ballast water treatment technologies, Hamburg, Germany.
- Shikuma, N. J., & Hadfield, M. G. 2010. Marine biofilms on submerged surfaces are a reservoir for *Escherichia coli* and *Vibrio cholerae*. *Biofouling*, 26, 39-46. doi: 10.1080/08927010903282814
- Urakawa, H., Kita-Tsukamoto, K., Steven, S. E., Ohwada, K., & Colwell, R. R. 1998. A proposal to transfer *Vibrio marinus* (Russell 1891) to a new genus *Moritella* gen. nov. as *Moritella marina* comb. nov. *FEMS Microbiology Letters*, 165(2), 373-378. doi: 10.1111/j.1574-6968.1998.tb13173.x



Photo: Reidun Kraugerud ©Nofima

FLEXIBAG - Water quality in Flexibag semi-closed system for post-smolts

Project leader: Jelena Kolarevic, Nofima
R&D partner: Bendik Fyhn Terjesen, Steve Summerfelt
User partner: Smøla Klekkeri og Settefisk

Background

A number of uniquely designed semi-closed containment concepts are currently being tested by the Norwegian aquaculture industry. A floating semi-closed containment system (S-CCS) with flexible walls made of tarpaulin was tested for the first time at the commercial scale during summer 2014, at Smøla (location Gullklakken). A high survival rate (92%) and specific growth rate (2.2%/day) of Atlantic salmon post-smolts were documented during two months long production cycle. However, the testing of the first generation of flexibag indicated challenges with fish transfer, water flow, oxygenation, particle collection and water velocity in the bag that have to be further addressed before the system can be used at its maximum desired and dimensioned capacity.

A second, improved generation of the flexibag was constructed and was tested in collaboration with SINTEF Fiskeri og Havbruk and Nekton at Smøla in the summer of 2015 as a part of the CtrIAQUA project "FLEXIBAG".

The main objective of this project was to

monitor the water quality in the 2nd generation of the flexibag. Therefore, point measurements of water quality parameters have been done within the project in order to document the water quality within the flexibag. The results on vertical and horizontal water quality profile will be presented in this deliverable. In addition a short summary of system and fish performance will be given.

Material and methods

The technical specification of the 2nd generation of the flexibag (Fig.5.19) is given in Table 5.5. The detailed description of the flexibag concept is given in Kolarevic et al. (2015). For the 2nd generation of the flexibag the volume of the bag was increased by 780 m³ and with it the maximum biomass capacity was increased from 75000 to 130910 kg. The pump capacity remained the same, increasing the retention time in the bag from 45 to 66 minutes. The buoyancy of the bag was improved by installation of additional float under the ring of the bag.

The installation started in April 2015 and by mid-May (Fig.5.20). Total of 111399 smolts with the average weight of 98g were transported from the land based facility to Gullklakken on 16.06.2015. Transfer of fish to the flexibag was done directly from the truck with a 100 m long pipe with a diameter of 160 mm. Fish were checked for lice every two weeks and regular veterinary visits were done during production period.

The experimental and production period in the flexibag lasted for 44 days. Nekton Havb-

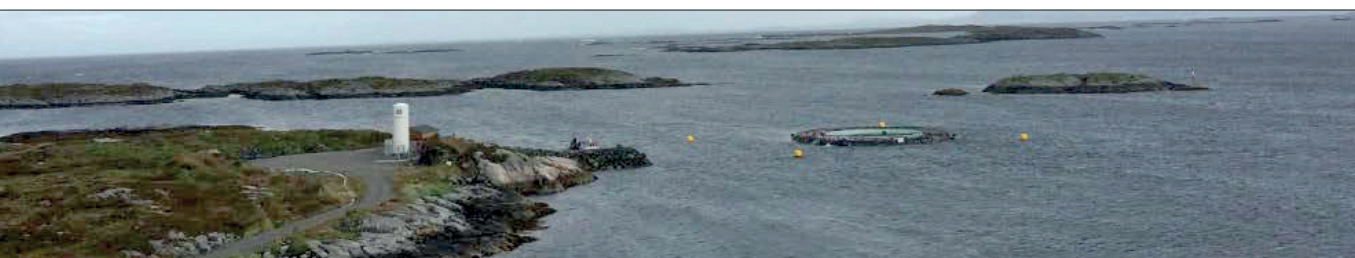


Fig. 5.19 Flexibag with the surrounding 60m sea cage at Gullklakken, Smøla (photo by Jelena Kolarevic, Nofima)

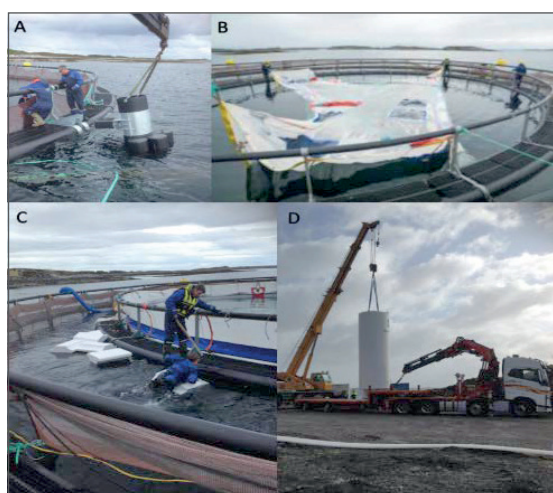


Fig.5.20 Installation of the intake pumps (A), bag (B), additional floats (C) and the oxygen tank (D) (photos by Rune Iversen, Nekton AS).

Table 5.5. Technical specification of the 2nd generation of the flexibag, based on rated equipment capacities and production and waste metabolite modelling.

Parameters	Values and units
Water volume	2500 m ³
Water exchange	38 m ³ /min
Specific pump capacity	158 l/sec per pump
Hydraulic retention time (HRT)	66 minutes
Minimum specific water flow (model)	0.29 l/min/kg
Maximum fish density (model)	52 kg/m ³
Maximum biomass (model)	130 910 kg
Maximum oxygen added (model)	663 kg/day
Maximum CO ₂ in the outlet water (model)	8 mg/L

ruk measured oxygen saturation and temperature on a daily basis during the trial. In addition, Nofima sampled water and measured concentration of CO₂, dissolved oxygen, temperature, pH, salinity, conductivity, turbidity, total ammonia nitrogen (TAN), total suspended solids (TSS), filtered total organic carbon

(TOC) within the semi-closed system, and in the surrounding water on two occasions (15.07 and 27.07.2015) for more detailed water quality analysis. Water was taken from the tank center at three different depths: 1.5m, 4m and 8m. In addition, measurements were done in the vicinity of one of the outlets at following depths: 1 m, 4m and 8m and in one of the inlets (at 1.5m) and in the surrounding seawater outside the bag (at 1.5m).

Results and Discussion

The prototype of the flexibag for production of post-smolt was tested in 2014 at Smøla. Fish performance was satisfactory with 92% survival rate, specific growth rate of 2.2% and absence of lice (Kolarevic et al., 2015). However, a number of challenges that were related to operation, water quality and fish welfare were underlined (Kolarevic et al., 2015). In order to address those issues further development of this concept resulted in 2nd generation of the flexibag that was tested at the same location in summer 2015.

The volume of the bag was increased in order to come closer to the standard net pen size and capacity. After the bag was mounted and filled with water it had conical shape that remained even when the pump were stopped for a week. The loss of volume in this period was minimal. Additional floaters were installed to improve buoyancy of the bag and increased oxygenation capacity were just some of the changes/improvements that were done.

Biofouling was a concern during the first production cycle, both inside and outside of the bag, with large numbers of tunicates forming colonies on the outside of the bag and increasing the weight of the whole system (Martinsen, 2015). Therefore, special attention was given to keeping the bag clean and two rounds of cleaning inside and outside were done during production cycle this year. While

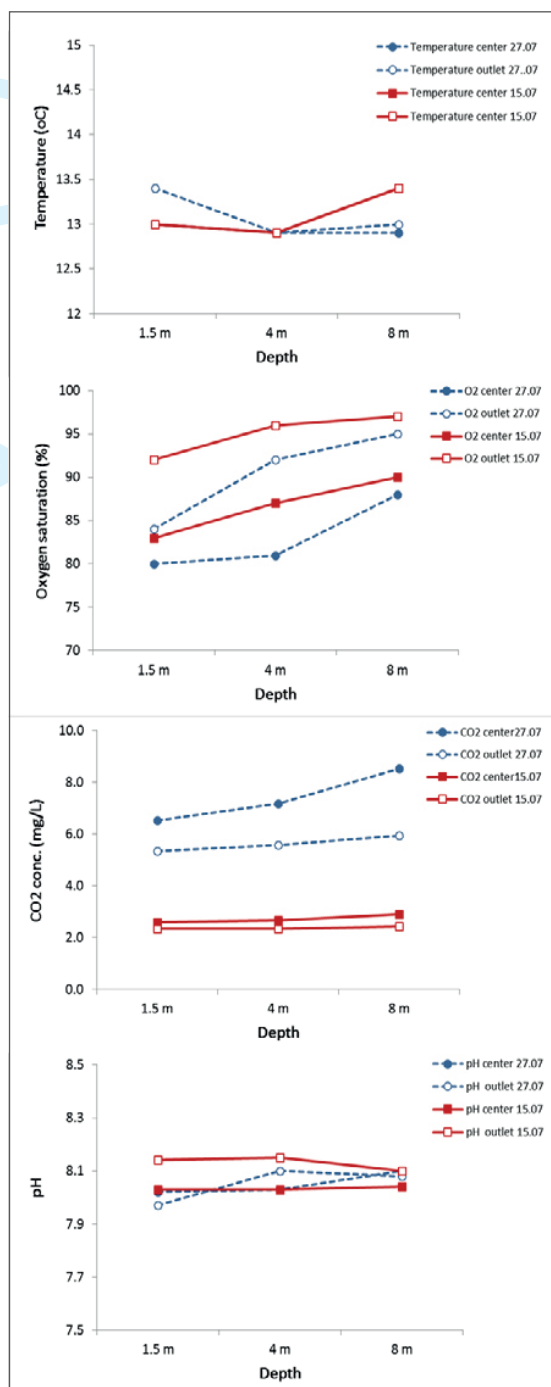


Fig.5.21 Water quality parameters measured at the bag outlet and in the center of the flexibag at 3 depths: temperature (A), oxygen (B), CO₂ (C), pH(D). The measurement done on the 15.07 are presented with red full squares (center of the bag) and with red empty squares (outlet) and connected with the red lines. The measurement done on the 27.07 are presented with blue full squares (center of the bag) and with blue empty squares (outlet) and connected with the blue dotted lines)

the cleaning inside the bag was successful, the cleaning rigs were not able to follow the conical shape of the bag outside all the way to the bottom and the oxygen supply tubes on the bag's flexible walls also presented obstacle for efficient cleaning. Water velocity in the upper part of the water column was characterised as satisfactory, however the removal of sludge and dead fish from the bottom of the bag proved to be challenging due to the low water velocity in this area. Self-cleaning has improved by opening number of the bottom hatches and by regular diver inspections. The possibility for continuous flow of water from the bottom of the bag that would amount to the 10% of the total flow in the bag was discussed as a solution that could improve both self-cleaning and the water flow in this area.

The transfer of fish to the bag was done directly from the truck and was satisfactory. In the first week after the transfer survival rate in the bag was around 97%. However, the delivery of the fish to the well boat presented a challenge as in the first round of testing due to the presence of the outer net pen that is still being used as a "safety" net while the flexibag system is being certified. The small but noticeable % of the mortality among post-smolts that was caused by presence of the outer net is a concern when the fish welfare is in question. At the end of testing period the survival rate was 95.3%.

The production cycle in the flexibag lasted for only 44 days, partly due to technical reasons and partly due to the concern about possible outbreak of AGD that occurred in the previous testing period (Kolarevic et al., 2015). The initial density at the start of the experiment was 4.4 kg/m³ while at the end of the experiment it increased to 6.5 kg/m³. Although the fish showed good appetite the SGR in this period was at 1% day⁻¹ indicating that fish might have undergone an adaptation period in which the feed intake and growth might have been reduced.

Indeed, a decrease in feed intake is observed for salmon after transfer to sea (Alne et al., 2010). In addition the feeding frequency and the feed distribution in the bag might not have been optimal and should be taken into consideration in the future.

Sea lice was not observed in the flexibag during production cycle in two consecutive years. This indicates that the concept of SCCS is justifying one of its main purpose namely the reduction or the elimination of lice from the salmon production systems in the sea.

Water quality in the flexibag during the production cycle was satisfactory and none of the measured parameters reached known toxic concentration for Atlantic salmon (Fig.5.21). The presence of solids in the bag was visible as the number of water quality parameters related to the particulate matter (TSS, turbidity, TOC) were higher in the bag compared to the surrounding seawater. Temporal analysis of the water quality in the bag showed the significant deterioration of number of parameters within 12 days of production. Parameters indicative of the particulate content were particularly affected. The reason for this observation could be an increase in feeding and the biomass in the bag and insufficient self-cleaning observed on the bottom of the bag close to the center of the bag.

In addition, significant differences were found in the horizontal profile of the water quality on both sampling dates. Water in the center of the bag had lower oxygen saturation, lower pH and higher CO₂ concentration compared to the water close to the one of the outlets that could indicate the presence of less mixed water in this area.

Conclusions

In conclusion, a number of improvements have been made in the bag's design and management. Atlantic salmon post-smolt has been produced at high survival rate and no lice was found in the bag.

However, due to the short production cycle the final density in the bag did not exceed 13% of the maximum density the system was designed for. And although the water quality in the flexibag in general was satisfactory, the significant difference observed in a number of parameters in less than 2 weeks of production at the low biomass and density (compared to the max. values bag is designed for) shows the need for further monitoring at more intensive production conditions.

Based on the experience from the daily management of the bag and based on the observed difference in the horizontal profile of the water quality, self-cleaning of the bag should be improved, potentially by providing more flow in the bottom of the bag.

In order to further develop the flexibag concept and to test its performance at higher biomass load and over longer period of time another round of production in the flexibag is starting in December 2015 and will continue into 2016. The water quality will be monitored as a part of the FLEXIBAG project in CtrlAQUA and as a continuation of this project into 2016.

References

- Alne, H., Oehme, M., Thomassen, M., Terjesen, B., Rørvik, K.A., 2010. Reduced growth, condition factor and body energy levels in Atlantic salmon (*Salmo salar* L.), during their first spring in the sea. *Aquaculture Research*. doi:10.1111/j.1365-2109.2010.02618.x.
- Kolarevic, J., Martinsen, S., Iversen, R., Terjesen, B.F. 2015. Testing of a semi-closed containment system for production of Atlantic salmon postsmolts (OPP5b experiment at Smøla Klekkeri og Settefisk and Nekton Havbruk, Smøla). *Nofima Report K-7/2015*, p.18
- Martinsen, S. 2014. *Helduk og not i lukket anlegg (HDN). Presentation at the 3rd conference on recirculation of water in aquaculture, Sunndalsøra, 22.-23.10.2014.*

PRELINE - Documentation of post smolt welfare and performance in large scale Pre-line semi-containment system (CCS)

*Project leader: Sigurd Handeland, UNI
R&D partner: Marco Vindas, Tom Nilsen, Lars Ebbesson, Sven Martin Jørgensen and Sigurd Stefansson, Cindy Pedrosa and Are Nylund
User partner: Lerøy Seafood Group*

Introduction

The production of Atlantic salmon post-smolts has highest losses, about 20 % of the fish, after seawater transfer (FKD 2004, 2011; Gullestad et al., 2011). Therefore, prolonging the time fish spend in land-based recirculation systems or in semi-closed rearing systems in sea, before transfer to sea cages, is expected to produce a larger and more robust fish (Kjartansson et al. 1988, Rosten et al. 2011, Thorarensen and Farrell, 2011, Terjesen et al. 2013). The semi-closed system called Preline takes water from 35 m deep water to create a constant laminar water flow at the surface. This semi-closed raceway concept has the potential of minimising pathogen infection, such as salmon lice, in the fish as well as reducing the risk of farmed escapees. In addition, fish welfare may be potentially boosted by inducing improved cardiac health, immune response, behaviour, product quality, osmoregulation and overall body composition by means of increased aerobic training (*i.e.* swimming due to the constant current

(Palstra 2012, Castro et al. 2013, Magnoni et al. 2013, Osachoff et al. 2014). The Preline experiment was conducted under normal industry aquaculture conditions in order to gain more knowledge on important production parameters, such as growth, condition, welfare, health condition, swimming behaviour, muscle fibre density and of Atlantic salmon post-smolts reared in this system.

Materials and Methods

The Preline semi-closed raceway system was located at Sagen II in the Trengereid fjord (Fig 5.22A). A traditional open circular 120 m sea cage was used as control and was located at Rongøy, Øygarden (Fig 5.22B). The Preline platform is 50 m long and holds approximately 2000 m³ water volume and collects water from a depth of 35 m and circulated via the inlet pipe to the outlet pipe to form a laminar one-way current through the system (Fig 5.22C). Oxygen concentrations and feeding were controlled by automatic systems and all data was registered daily. Throughout the experimental period, both the Preline and control cage were fitted with underwater video cameras in order to monitor fish behaviour, general water conditions, mortality and appetite.



Figure 5.22. A. Preline location at Sagen II, Trengereid fjord. B. Control sea cage facilities. C. Diagram of the Preline semi-closed system.

Experimental protocol

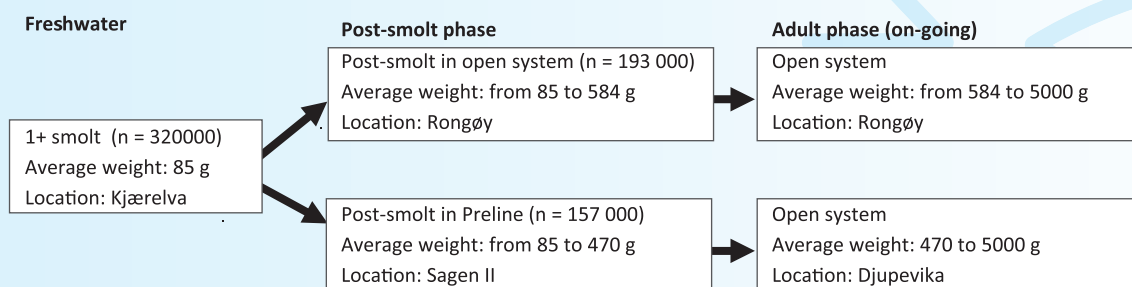


Figure 5.23. Schematic representation of the experimental design.

1. Freshwater phase sampling

There were 3 samplings conducted during the freshwater phase. Size (weight in g and fork length in mm), condition, welfare, smolt development and quality were measured for all three sampling points.

2. Post-smolt phase sampling

Two samplings were conducted during the postsmolt phase. Fish were randomly collected, measured and sampled for muscle fiber density, as well as gills, heart and head kidney in order to control for pathogen abundance, quality and welfare indicators. In addition, water quality measurements were also taken twice during the experiment.

Results and Discussion

Smolt quality: In salmonids, successful transfer from freshwater into seawater is associated with the increase of the sodium, potassium ATPase ($\text{Na}^+\text{-K}^+\text{-ATPase}$) enzyme activity, which is found in gill chloride cells (Nilsen et al. 2007, 2008). Our results show that all individuals showed optimal smolt-quality before seawater transfer.

Water quality: The salinity, carbon dioxide (CO_2) and total ammonia nitrogen concentrations were higher at the Preline system, compared to the control facilities, but were within accepted parameters. The water temperature was lower at Preline, compared to control facilities. This temperature difference was

the result of water taken from a 30 m depth at the Preline facilities, while at the control farm, fish had access to warmer surface water throughout the summer.

Growth rate and feed conversion ratio (FCR): Preline fish had lower overall weight (470 g) at the end of the summer, compared to control individuals (584 g). The FCR was lower (0.81) in Preline fish, compared to control (1.09). Since temperature and growth rate are correlated, it is likely the different water sources generated the growth differences observed at the two facilities (Solbakken et al. 1994, McCormick et al. 2002, Handeland et al. 2004, 2008).

Survival, health and welfare status: while mortality increased at control facilities throughout the experiment (from 1.2 to 4 %), it was maintained constant at 1.2 % at Preline facilities during the whole experimental period. Furthermore, while control fish presented an incidence of sea lice and amoebic gill disease (AGD), which required treatment, this was not the case in Preline fish. The increased survival in Preline fish was likely associated with higher exposure of pathogens at the control facilities. These results therefore suggest a general higher health status for Preline fish compared to control.

Swimming behaviour: Preline fish remained more or less at the same location in the water swimming against the current (similarly to

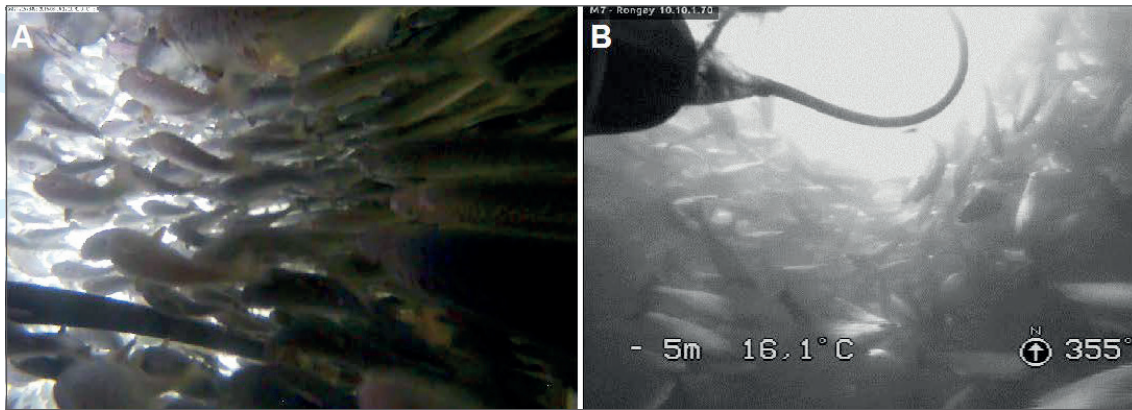


Figure 5.24. Picture frame from underwater video recording showing the general swimming pattern of fish at the Preline (A) and control (B) facilities.

wild fish in a river), while control individuals swam in no discernible patterns throughout the fish cage (Fig 5.24).

Muscle fibre analysis: There was a general tendency for a higher number of small fibres (20-40 and 40-60 μm) in fish of both treatment groups (Figure 5.25). However, the Preline fish had 3.8 times higher amounts of fibres ranging from 0-20 μm , compared to control individuals. This result may be indicative of a higher production/recruitment

of muscle fibres (Castro et al. 2013, Osachoff 2014). Notably, actively swimming against the current at Preline facilities implies a higher exercise regime compared to fish kept in the sea cage system (with no laminar flow).

We therefore hypothesize that Preline fish, exposed to an increased training regime, promote higher muscle growth than fish not exposed to this regime. Further, higher aerobic exercise has been associated with several benefits, such as increased cardiac health and

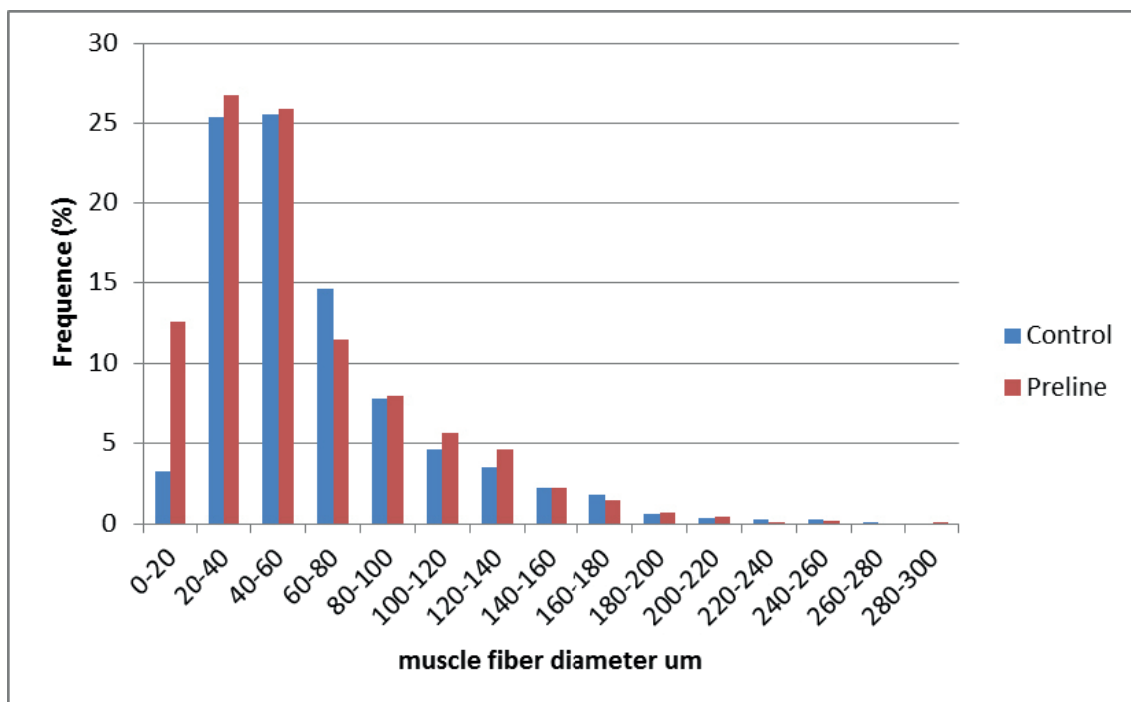


Figure 5.25. Distribution of muscle fiber size in post-smolt salmon at Preline and control facilities.

better immune response (Castro et al. 2013, Magnoni et al. 2013, Osachoff 2014), so the potential additional benefits of the laminar flow of the Preline system enhance the welfare environment. Testing this hypothesis and exploring possible implications for Preline fish along production up to slaughter, will be the aim of on-going adult seawater experiments.

References

Castro, V., Grisdale-Helland, B., Helland, S.J., Torgersen, J., Kristensen, T., Claireaux, G., et al. 2013. Cardiac Molecular-Acclimation Mechanisms in Response to Swimming-Induced Exercise in Atlantic Salmon. *PLoS ONE* 8(1): e55056. doi:10.1371/journal.pone.0055056.

FKD, 2004. Merknader til Forskrift 22. desember 2004 nr. 1785 om drift av akvakulturanlegg (akvakulturdriftsforskriften, In Norwegian).

FKD, 2011. Høring av forslag om økt individvekt for settefisk av laks, ørret og regnbueørret. Fiskeri- og Kystdept., Oslo. (In Norwegian).

Gullestad, P., Bjørge, S., Eithun, I., Ervik, A., Gudding, R., Hansen, H., Johansen, R., Osland, A., Rødseth, M., Røsvik, I., Sandersen, H., Skarra, H., Bakke, G., 2011. Effektiv og bærekraftig arealbruk i havbruksnæringen (In Norwegian, "Efficient and sustainable use of areas in Norwegian mariculture"), Oslo, pp. 190. (In Norwegian).

Handeland, S. O., Imsland, A. K. and S. O. Stefansson. 2008. The effect of temperature and fish size on growth, feed intake, food conversion efficiency and stomach evacuation rate of Atlantic salmon post-smolts. *Aquaculture*. 283, 36-42.

Handeland, S. O., Wilkinson, E., Sveinsbø, B., McCormick, S. D. & Stefansson, S. O. 2004. Temperature influence on the development and loss of seawater tolerance in two fast

growing strains of Atlantic salmon. *Aquaculture* 233, 513-529.

Kjartansson, H., Fivelstad, S., Thomassen, J.M., Smith, M.J., 1988. Effects of different stocking densities on physiological parameters and growth of adult Atlantic salmon (*Salmo salar* L.) reared in circular tanks. *Aquaculture*. 73, 261-274.

Magnoni, L. J., Crespo, D., Ibarz, A., Blasco, J., Fernández-Borràs, J. and J. V. Planas. 2013. Effects of sustained swimming on the red and white muscle transcriptome of rainbow trout (*Oncorhynchus mykiss*) fed a carbohydrate-rich diet. *Comparative Biochemistry and Physiology, Part A* 166. 510-521.

McCormick, S.D., Shrimpton, J.M., Moriyama, S., Björnsson, B.T. 2002. Effects of an advanced temperature cycle on smolt development and endocrinology indicate that temperature is not a zeitgeber for smolting in Atlantic salmon. *J Exp Biol* 205, 3553-3560.

Nilsen, T. O., Ebbesson, L. O. E., Kiilerich, P., Björnsson, B. Th., Madsen, S. S., McCormick, S. D. & Stefansson, S. O. (2008). Endocrine systems in juvenile anadromous and landlocked Atlantic salmon (*Salmo salar*): seasonal development and seawater acclimation. *General and Comparative Endocrinology* 55, 762-772.

Nilsen, T.O., Ebbesson, L.O.E., Madsen, S.S., McCormick, S.D., Andersson, E., Björnsson, B.T., Prunet, P., Stefansson, S.O., 2007. Differential expression of gill Na⁺,K⁺-ATPase α - and β -subunits, Na⁺,K⁺,2Cl⁻ cotransporter and CFTR anion channel in juvenile anadromous and landlocked Atlantic salmon *Salmo salar*. *Journal of Experimental Biology*. 210, 2885-2896.

Osachoff, H. L., Osachoff, K. N., Wickramaratne, A. E., Gunawardane, E. K., Venturini, F. P. and C. J. Kennedy. 2014. Altered burst swimming in rainbow trout *Oncorhynchus mykiss* exposed to natural and synthetic oestro-

Palstra, A. J. 2012. *Swimming Physiology of Fish: Towards Using Exercise to Farm a Fit Fish in Sustainable Aquaculture*. ISBN: 978-3-642-31048-5 (Print) 978-3-642-31049-2 (Online) pp 430.

Rosten, T.W., Ulgenes, Y., Henriksen, K., B.F., T., Biering, E., Winther, U., 2011. *Oppdrett av laks og ørret i lukkede anlegg - forprosjekt, Utredning for Fiskeri og havbruksnæringens forskningsfond (FHF)*. (In Norwegian).

Solbakken, V.A., Hansen, T., Stefansson, S.O., 1994. *Effects of photoperiod and temperature on growth and parr-smolt transformation in Atlantic salmon (Salmo salar L.) and subsequent performance in seawater*. *Aquaculture* 121, 13–27.

Terjesen, B., Rosten, T., Ulgenes, Y., Henriksen, K., Aarhus, I., Winther, U., 2013. *Betydning av vannmiljøet ved produksjon av laksefisk i lukkede systemer i sjø. Water quality requirements for efficient farming of Atlantic salmon in closed systems. In Norwegian, English abstract*. VANN. 48, 14–27.

Thorarensen, H., Farrell, A., 2011. *The biological requirements for post-smolt Atlantic salmon in closed-containment systems*. *Aquaculture*. 312, 1–14.

ROBUST - Robustness evaluation parameters associated with biological requirements in closed systems

Project leader: Lars Ebbesson, UNI Research

R&D partners: Sigurd Handeland, Tom Ole Nilsen, Marco Vindas, Simon Mackenzie, Bendik Fyhn Terjesen, Jelena Kolarevic, Sven Martin Jørgensen, Sara Calabrese, Valentina Tronci

User partners: Marine Harvest

Introduction

Robustness is a central theme in the Department of Fish Production and Welfare in CtrIAQUA. How the intensive chronic closed-containment systems may affect the ability of postsmolts to respond to new acute challenges, such as confinement or subsequent transfer to sea cages is unresolved. Knowing the environmental and physiological limits will avoid situations that reduce the fish's capacity to respond to new challenges and compromise welfare (Ebbesson and Braithwaite 2012). While physiological homeostasis can be maintained under chronic mild stress, an additional challenge can push the animal over to allostatic overload, resulting in compromised welfare, through physiological and cognitive dysfunction or lack of response (Korte et al 2007, Grassie et al., 2013). We hypothesise that this acute challenge test approach is more sensitive to discover latent welfare and robustness issues, than acute or chronic studies alone.

We previously conducted experiments with postsmolts investigating fish density limits. In these experiments, growth, endocrine, physiological, and external welfare parameters led to important conclusions (Calabrese et al submitted). These data showed that salmon that were raised in a density of 75 kg/m³ demonstrated good physiology and welfare, whereas fish at 100 kg/m³ and 125 kg/m³ showed decreased welfare and health. Circulating cortisol levels, a common measure of stress, were however difficult to interpret. In all densities, acute challenged fish showed an increase in cortisol, although the 125 kg/m³ group showed less up-regulation. In addition, the 25 kg/m³ group showed that one replicate had increased cortisol levels and assumed that this was a tank disturbance just prior to sampling (cortisol release is very sensitive to external tank disturbances and increase in the plasma within 5 min). This replicate was subsequently removed from analysis. Recent studies have shown that the measure of stress

with cortisol can be misleading and is not always a good predictor of functional output, as learning and adaptation (Grassie *et al* 2013, L Ebbesson unpublished observations). To identify welfare indicators that can predict good and poor welfare situations reliably, we have targeted newly characterized mental robustness indicators that link stress responses to learning and memory, essential for coping and adapting to changing environments (Ebbesson and Braithwaite, 2012; Grassie *et al.*, 2013; Salvenes *et al.*, 2013, Braithwaite and Ebbesson, 2014; Madaro *et al.*, 2015; Manuel *et al.*, 2015).

Aim, Results and Discussion

The aim of ROBUST was therefore to further evaluate environmental and biological requirements investigated in the Department of Fish Production and Welfare by further anal-

ysis of mental robustness in brain samples in experiments associated with maximizing fish density while maintaining good growth and welfare.

Our results show that the 75 kg/m³ are able to up-regulate important responses in the neural stress axis and neural plasticity markers, while in groups that are previously stressed cannot activate appropriate neural responses when challenged. These data are in agreement with previous studies in salmon (Grassie *et al.*, 2013, Madero *et al* 2015) and further suggest that mental robustness indicators will be of value in interpreting environmental requirements for CCS and SCCS.

References

Braithwaite, V.A. and Ebbesson, L.O.E. 2014. Pain and stress responses in farmed fish. In:

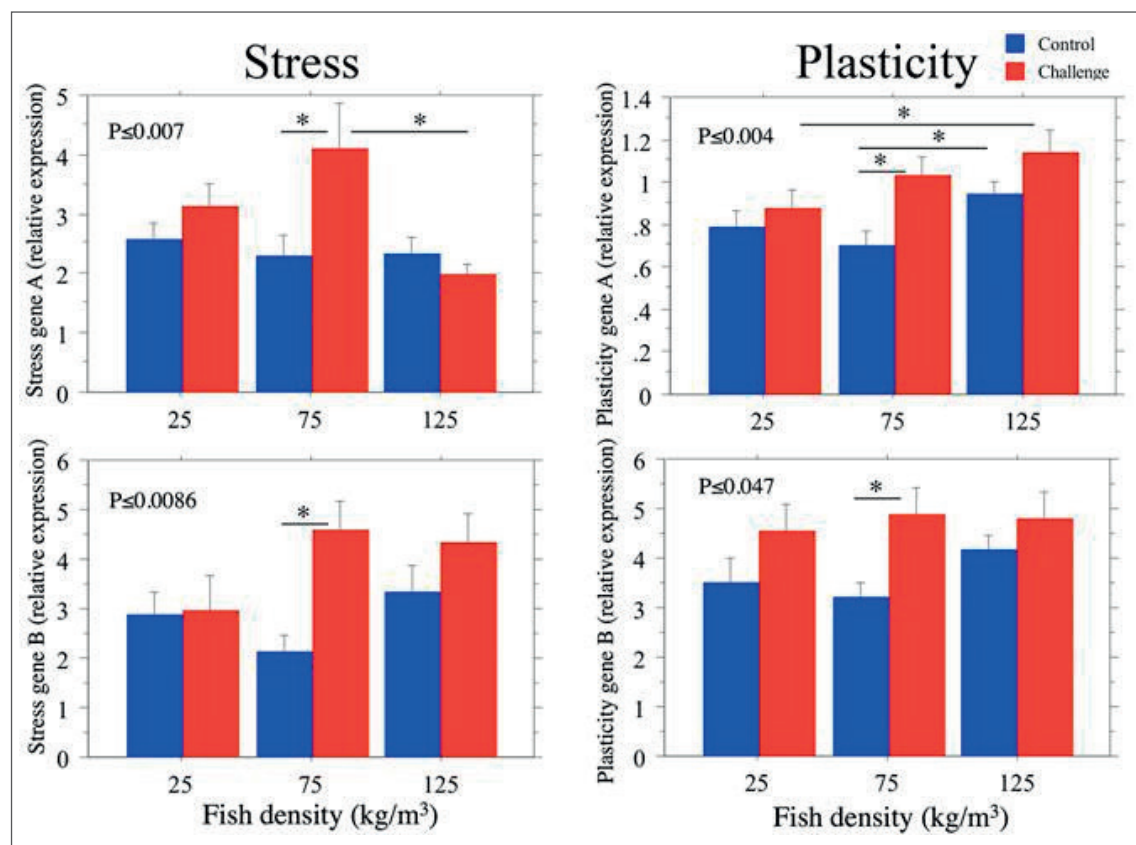


Figure 5.26. Expression of stress and neural plasticity mRNA levels in the forebrain of Atlantic salmon reared in different densities for 8 weeks. Fish raised at 75 kg/m³ were able to activate appropriate neural systems to respond to stress that facilitate learning and adaptation. Control fish (blue bars) and fish that were exposed to an acute challenge test (red bars).

Animal Welfare: focusing on the future. OIE Scientific and Technical Review, 33, 245-253.

Ebbesson, L.O.E. and Braithwaite, V.A. 2012. Environmental impacts on fish neural plasticity and cognition. *Journal of Fish Biology* 81, 2151-2174.

Grassie, C., Braithwaite, V.A., Nilsson, J., Nilsen, T.O., Teien, H.C., Handeland, S.O., Stefansson, S.O., Tronci, V., Gorissen, M., Flik, G., Ebbesson, L.O.E. 2013. Aluminum exposure impacts brain plasticity and behavior in Atlantic salmon (*Salmo salar*). *Journal of Experimental Biology*, doi:10.1242/jeb.083550

Korte, S.M., Olivier, B., Koolhaas, J.M., 2007. A new animal welfare concept based on allostasis. *Physiology & Behavior* 92, 422-428.

Madaro, A., Olsen, R.E., Kristiansen, T.S.,

Ebbesson, L.O.E., Nilsen, T.O., Flik, G., Gorissen, M. 2015. Stress in Atlantic salmon: response to unpredictable chronic stress. *J Experimental Biology*, doi: 10.1242/jeb.120535
Manuel, R., Gorissen, M., Zethof, J., Ebbesson, L.O.E., Vis, H., Flik, G., Bos, R. 2014. Unpredictable chronic stress decreases inhibitory avoidance learning in Tuebingen Long-Fin zebrafish (*Danio rerio* Hamilton): stronger effects in the resting phase than in the active phase. *J Experimental Biology*, DOI: 10.1242/jeb.109736.

Salvanes, A.G.V., Moberg, O., Ebbesson, L.O.E., Nilsen, T.O., Jensen, K.H., Braithwaite, V.A. 2013. Environmental enrichment promotes neural plasticity and cognitive behaviour in fish. *Proceedings of the Royal Society B* doi:10.1098/rspb.2013.1331

Wendelaar Bonga, S.E., 1997. The stress response in fish. *Physiol Rev.* 77, 591-625.

6. International cooperation

In CtrlAQUA, extensive international scientific collaborations are ongoing. Among these the following partners are of particular importance for the center:

1. University of Gothenburg (UGOT):

Gothenburg University is represented in CtrlAQUA by Prof. Kristina Sundell and her research group. In CtrlAQUA UGOT contributes to most of the projects in Dept. Fish Prod & Welfare and Dept. Prev Fish Health. In the current reporting period UGOT participated in the project "Osmoregulatory barrier function in post-smolts reared in closed-containment systems (BARRIER)".

2. The Conservation Fund Freshwater Institute (FI):

The Conservation Fund Freshwater Institute (FI) is represented in CtrlAQUA by Dr. Steven Summerfelt, Dr. Brian Vinci, and Dr. DVM Chris Good. FI contributes to the

projects in the departments Dept. Tech & Env. and Dept. Prev Fish Health. In the current reporting period, FI led the project "Hydrodynamic challenges in huge tanks (1000+ m³) (HYDRO)". They also participated in the projects "Osmoregulatory barrier function in post-smolts reared in closed containment systems (BARRIER)", "Review of pathogens representing a particular risk in closed containment systems (RISK)", "Particle and pathogen removal from intake water in semi-closed systems (INTAKE)", and "Particle tolerance in post-smolts reared in RAS (PARTICLE)".

3. International collaboration was also done in the project "Particle tolerance in post-smolts reared in RAS (PARTICLE)" with Aquantis, a German subsidiary of Veolia, in which CtrlAQUA partner Kruger Kaldnes also is a subsidiary.

7. Recruitment

According to the proposal and contract, a total of 15 PhD students will be educated through the life-time of CtrlAQUA, and enrolled at the University of Bergen and NTNU. From the beginning of the centre period, we have been recruiting PhD students to central research topics of the centre. The requirement by the Research Council to prepare annual plans for SFI's can, however, come into conflict with the requirements of the PhD-programs at the two universities, since the students are obliged to plan ahead for three to four years. As was shown in the introduction to this annual report however, we plan to submit proposals for annual projects

along several long-term research lines. These research lines will serve as anchors to develop PhD plans of sufficiently long duration and predictability. The CtrlAQUA leader group, the Board and the user partners will consider this need for long-term planning of PhD's, in their priority of projects each year.

In addition to PhD fellows, CtrlAQUA seeks to educate a number of Master students, at the University of Bergen, Göteborg University and NTNU (see table 7.1). Several candidates are already doing their theses in CtrlAQUA projects.

Table 7.1. Post-docs, PhD's and master students currently attached to CtrlAQUA

Partner	Candidate	Funding	Project association	2015	2016	2017	2018	2019	2020	2021	2022	2023
PhD-students												
Marine Harvest	Sara Calabrese	Industry-PhD	OPP									
Nofima	Lene Sveen	RCN	SalmoFutura									
UiB	Victoria Røyseth	UiB	MICROPARASITES									
NTNU	Xiaoxue Zhang	RCN	SENSOR									
Post-docs												
ORP	Nhut Tran-Minh	RCN Havbruk	POCNAD									
MSc students												
UGOT	Britt Sjöqvist	RCN	BARRIER									
UGOT	Ida Heden	RCN	BARRIER									
UiB	Øyvind Moe	RCN	PRELINE									
UiB	Ingrid Gamlem	RCN	PRELINE									
UiB	Egor Gaidukov	RCN	TRANSFER									

8. Communication and dissemination activities

In CtrlAQUA, the overall goal with communication is to create interest around the activity of the center, and to be a strategic contribution to attain the goals of CtrlAQUA. The communication shall mirror the vision of the center. Therefore, a common basis for profiling the center was important for this starting year of CtrlAQUA. A communication plan and profile handbook with logos was made early in this process.

When it comes to internal routines and systems for communication between the partners, the intranet is the most important. The intranet is the main communication channel within the center for the 85 participants now involved. The intranet has a document base, image base, message facilities, calendar and internal alerts of new findings or publications as agreed upon in the consortium agreement. Other systems for internal communication are regular meetings, and providing instructions for presenting CtrlAQUA.

The main external communication channel is the website www.ctrlaqua.no, which is well designed for presenting results, activities, publications and innovations as the centre develops. Also, templates for presentations, roll

up, folder, and fact sheet have been made to show externally what the center is about.

The interest from industry, the public and academia has been great in the start-up phase of the center. This has resulted in 44 news articles in press in 2015, mostly generated by media itself. In addition, research partners have actively made news stories covered in professional media, and all partners have been available for press to report on the progress of research and innovation in CCS in aquaculture.

Examples of dissemination activities in 2015 are:

- Attention from the general public around the visit from Minister of Fisheries during kick-off
- Several presentations during the industry fair AquaNor 2015
- Information about research on closed-containment aquaculture at Forskningstorget i Oslo 2015, directed at children, families and schools
- Dissemination towards a scientific audience at the 3rd Nordic RAS workshop in Molde



Figure 8.1. Cermaq and Nofima informed the public, mostly represented by school children, about the work being done in CtrlAQUA, at Forskningstorget in Oslo in September 2015.

Attachments to the report:

Attachment 1: Personnel

Table 1. Key Researchers at the R&D partners in 2015

Name	Institution
Bendik Fyhn Terjesen	Nofima AS
Jelena Kolarevic	Nofima AS
Astrid Buran Holan	Nofima AS
Åsa Maria Espmark	Nofima AS
Sven Martin Jørgensen	Nofima AS
Turid Synnøve Ås	Nofima AS
Christian Karlsen	Nofima AS
Vasco Mota	Nofima AS (since December 2015)
Jagan Gorle	Nofima AS (since December 2015)
Lars Ebbesson	UNI Research
Sigurd Handeland	UNI Research
Tom Ole Nilsen	UNI Research
Sigurd Stefansson	University of Bergen (UiB)
Are Nylund	University of Bergen (UiB)
Øyvind Mikkelsen	Norwegian University of Science and Technology (NTNU)
Kristina (Snuttan) Sundell	University of Gothenburg (UGOT)
Henrik Sundh	University of Gothenburg (UGOT)
Brian Vinci	Freswater Institute (FI), USA
Chris Good	Freswater Institute (FI), USA
Steve Summerfelt	Freswater Institute (FI), USA

Table 2. PhD students working on projects in the centre with financial support from other resources

Name	Funding	Period
Sara Calabrese	Marine Harvest/NCR	2013-2016
Lene Sveen	NCR	2014-2017

Table 3. PhD students working on projects in the centre

Name	Period
Victoria Røyseth	2016-2019
Xiaoxue Zhang	2016-2019

Table 4. Key personnel from user partners in 2015

Company	Name	Position
Bremnes Seashore AS	Geir Magne Knutsen	Farming Manager
Cermaq Norway AS	Olai Einen	R&D Manager
FishGlobe AS	Arne Berge	Director
Grieg Seafood AS	Frode Mathisen	Director Biological Performance and Planning
Lerøy Seafood Group ASA	Harald Sveier	Technical Manager
Marine Harvest ASA	Ragnar Joensen	Group Manager Technology
	Harald Takle	Fish Health Specialist
	Sara Calabrese	PhD student
Smøla Klekkeri og settefiskanlegg AS	Per Gunnar Kvenseth	Managing Director
Aquafarm Equipment AS	Atle Presthaug	CEO
Krüger Kaldnes AS	Marius Hægh	VP Aquaculture
	Yngve Ulgenes	R&D Manager
Oslofjord Ressurspark AS	Frank Karlsen	CSO
Storvik Aqua AS	Knut Måløy	Chairman
	Arve Tronsgård	CEO
Pharmaq analytiq AS	Siri Vike	General Manager
	Stian Nylund	R&D Manager
Pharmaq AS	Nils Steine	Technical Manager
	Karine Lindmo Yttredal	Manager Virus Technology

Attachment 2: Accounting

Table 5. Project Costs 2015 (All figures in 1000 NOK)

Item	Collaboration project *	Type of Research**	Incentive effect***	Host - Nofima	UNI Research	Universitetet i Bergen	Freshwater Institute	NTNU	Gjøteborgs Univ	Marine Harvest	Grieg Seafood	Leroy Seafood	Cermaq	Bremnes	Kruger	Kaldnes	Pharmaq	Analytiq	Pharmaq	ORP	Storvik Aqua	Smøla K&S	Aquafarm Equip	Total cost
Project 1 - ADM	Yes	F	0111	1072	8	73																		1152
Project 2 - DATABASE	Yes	F	1011	109	705																			814
Project 3 - BARRIER	Yes	F	1011	620	587	6			196															1410
Project 4 - SENSOR	Yes	F	1011					168																168
Project 5 - RISK	Yes	F	1111	90		33	62																	185
Project 6 - HYDRO	Yes	F	1111	986	215		337																	1539
Project 7 - PARTICLE	Yes	F	1111	973	319		32				152			59	393									1928
Project 8 - BIOMASS	Yes	I	1111	301																	130			430
Project 9 - REMOVAL	Yes	I	1111	128																	64			192
Project 10 - MICROPARASITES	Yes	F	1101	337	215	220											223	281	964					2240
Project 11 - INTAKE	Yes	F	1011	392									142										88	621
Project 12 - FLEXIBAG	Yes	I	1111	157																		421		578
Project 13 - PRELINE	Yes	I	1111	36	314						1658													2008
Project 14 - ROBUST	Yes	F	1110	29	306	6				118														459
																								0
																								0
SFI Equipment																								0
SFI Administration, see ADM																								0
Total budget				5229	2668	338	431	168	196	118	152	1658	142	59	393	223	281	964	194		421	88		13723

* Collaboration project: YES / NO.

** Type of Research: F= Fundamental research, I=Industrial Research

*** Incentive effect, 1 =Present, 0=Not present. First digit: New R&D activity triggered, Second digit: Increase in size of related R&D activity, Third digit: Enhanced scope of related R&D activity, Fourth digit: Increased speed in execution of related R&D activity

Table 6. Project Funding 2015 (All figures in 1000 NOK)

Item	Aid Intensity Limit****		Host - Nofima	UNI Research	Universitetet i Bergen	Freshwater Institute	NTNU	Gjøteborgs Univ	Marine Harvest	Grieg Seafood	Leroy Seafood	Cermaq	Bremnes	Kruger Kaldnes	Pharmaq Analytiq	Pharmaq	ORP	Storvik Aqua	Smøla K&S	Aquafarm Equip	Other Private funding	RCN Grant	Other Public funding		Total funding	Indirect state aid *****
Type of partner****			R	R	R	R	R	R	L	L	L	L	L	SME	SME	SME	SME	SME	SME	SME						
Project 1 - ADM	100		226	1	11				22	22	22	22	22	17	15	15	17	15	9	11	434	706	11		1 152	b,c
Project 2 - DATABASE	100		22	108					17	24	18	20	22	11	11	11	23	16	7	7	316	498	0		814	b,c
Project 3 - BARRIER	100		124	90	1			36	30	33	33	33	19	18	18	30	24	10	11	542	867	1		1 410	b,c	
Project 4 - SENSOR	100						58			1	1	1	1	1	1	1	1	1	1	10	100	58		168	b,c	
Project 5 - RISK	100		18		5	9				2	3	2	3	3	1	2	2	2	1	1	50	130	5		185	b,c
Project 6 - HYDRO	100		198	33		48			28	29	29	31	34	20	18	18	25	17	4	11	545	994	0		1 539	b,c
Project 7 - PARTICLE	100		195	49		5				196			88	590							1 123	805	0		1 928	b,c
Project 8 - BIOMASS	75		60															190			250	181	0		430	b,c
Project 9 - REMOVAL	75		26															89			115	77	0		192	b,c
Project 10 - MICROPARASITES	100		68	33	34										242	305	1 047				1 696	510	34		2 240	b,c
Project 11 - INTAKE	100		79									190								118	387	235	0		621	b,c
Project 12 - FLEXIBAG	75		30																454		484	94	0		578	b,c
Project 13 - PRELINE	65		7	48							1 738										1 794	214	0		2 008	b,c
Project 14 - ROBUST	100		6	47	1				194												247	210	1		459	b,c
SFI Equipment																										
SFI Administration, see ADM																										
Total budget			1 059	410	53	62	58	36	292	307	1 845	299	202	661	306	370	1 145	354	485	159	7 992	###	110		13 723	∞

**** Type of partner: R=Research Organization, P=Other public, L=Large Enterprise, SME=Small and medium sized enterprise

***** Aid Intensity Limit: Indicate percentage as follows: Fundamental research 100 %. Industrial research 65 % for collaboration projects, 75 % if only SMEs included in the collaboration project.

Attachment 3: Publications

Written publications:

Calabrese, S., Sævareid, B., Breck, O., Joensen, R., Nilsen, T.O., Kolarevic, J., Fiv-elstad, S., Takle, H., Hosfeld, C., Ebbesson, L.O.E., Stefansson, S., Terjesen, B.F., Handeland, S.O. 2015. Pilot study: Post-smolt Atlantic salmon production in semi-closed sea systems. In: Book of abstracts EAS Rotterdam 2015.

Holan, A.B., Kolarevic, J., Fossmark, R., Bakke, I., Vadstein, O., Terjesen, B.F. 2015. Evaluation of membrane treatment effect on water quality in recirculating aquaculture systems (RAS) for Atlantic salmon post-smolts (*Salmo salar*). In: Book of abstracts 3d Workshop on Recirculating Aquaculture Systems. p. 52. 30th of September 1st October 2015, Molde, Norway.

Holan, A.B., Kolarevic, J., Terjesen, B.F. 2015. Evaluation of membrane treatment in recirculating aquaculture systems (RAS) for Atlantic salmon post-smolts (*Salmo salar*). In: Book of abstracts EAS Rotterdam 2015.

Jørgensen, S.M. 2015. CtrlAQUA – Improving safe and sustainable production. Kaldnes RAS Magazine: INSIDE Aquaculture, Special Edition, AQUA NOR 2015.

Pautsina, A., Cisar, P., Stys, D., Terjesen, B.F., Espmark, Å.M. 2015. Infrared reflection system for indoor 3D tracking of fish. Aquaculture Engineering, 69, 7-17

Summerfelt, S.T., Mathisen, F., Holan, A.B., Terjesen, B.F. (Submitted-In Review). Survey of large circular and octagonal tanks operated at Norwegian commercial smolt and post-smolt sites. Aquacultural Engineering.

Sveier, H., Tangen, S., Handeland, S. 2015. Post smolt (*Salmo salar* L) production in floating raceway systems. In: Book of abstracts EAS Rotterdam 2015.

Terjesen, B.F. 2015. Nofima Center for Recirculation in Aquaculture (NCRA), a research facility for the future salmon production methods. Aquaculture Europe Magazine pp. 33-43.

Terjesen, B.F., Abbink, W., Blom, E., Kamstra, A., Espmark, Å.M., Kolarevic, J., Nilsen, T.O., Ebbesson, L., Handeland, S., Sveen, L., Takle, H. 2015. Scaling of culture tanks and unit processes, relevant for Atlantic salmon post-smolt production in land based systems. In: Book of abstracts 3d Workshop on Recirculating Aquaculture Systems. p. 52. 30th of September 1st October 2015, Molde, Norway.

Presentations (oral and posters):

Calabrese, S., Sævareid, B., Breck, O., Joensen, R., Nilsen, T.O., Kolarevic, J., Fiv-elstad, S., Takle, H., Hosfeld, C., Ebbesson, L.O.E., Stefansson, S., Terjesen, B.F., Handeland, S.O. 2015. Pilot study: postsmolt Atlantic salmon production in semi-closed sea systems. Aquaculture Europe, EAS, Rotterdam, The Netherlands, 20-23. October 2015.

Handeland, S., 2015. Produksjon av laks i lukkede anlegg. Får vi dette til? Hardangerfjordseminaret 2015.

Helland, S. 2015. Spennende satsing innen havbruk på Sunndalsøra: SFI CtrlAQUA. Den lukkede fasen i lakseoppdrett. Marin Samhandlingsarene. Ålesund, 25. November 2015.

Holan, A.B. 2015. Utviklingen innenfor bruk av RAS i norsk akvakultur. Farmer Day, AquaCircle, DanAqua, Aalborg, Danmark 7. October 2015.

Holan, A.B. Kolarevic, J., Terjesen, B.F. 2015. Evaluation of membrane treatment in RAS for Atlantic salmon post-smolts (*Salmo salar*). Aquaculture Europe, EAS, Rotterdam, The Netherlands, 20-23. October 2015.

Holan, A.B., Kolarevic, J., Fossmark, R.O., Bakke, I., Vadstein, O., Terjesen, B.F. 2015. Evaluation of membrane treatment effect on

water quality in RAS for Atlantic salmon post-smolts. NordicRAS Network, Molde Norway, 30. September - 1. October 2015.

Holan, A.B., Terjesen, B.F. 2015. CtrlAQUA Center for Closed Containment Aquaculture. Transatlantic Science week, The Norwegian Embassy in Washington DC, Ministry of Education and Research, Ministry of Trade, Industry and Fisheries, Research council of Norway. Boston, USA, 4-6. November 2015.

Sveier, H., Tangen, S., Handeland, S. 2015. Postsmolt of Atlantic salmon (*Salmo salar* L.) production in floating raceway system. Expectations and preliminary results. Aquaculture Europe, EAS, Rotterdam, The Netherlands, 20-23. October 2015.

Terjesen, B.F. 2015. Is the future land-based? Invited speaker and panel participant. Spare-Bank1 and BEWI seminar on salmon aquaculture. Trondheim, Norway, August 20th, 2015.

Terjesen, B.F. 2015. How do we farm salmon in the future? Sunndalskonferansen, Sunndalsøra, 23rd June 2015.

Terjesen, B.F. 2015. Can the salmon industry grow with closed-containment systems? Nofima AquaNor seminar. Trondheim, 18th August 2015.

Terjesen, B.F. 2015. Post-smolt production in closed-containment systems: CtrlAQUA SFI and other relevant RCN projects. The Research Council of Norway AquaNor exhibition stand. Trondheim, 19th August 2015

Terjesen B.F. 2015. CtrlAQUA – Centre for Closed-Containment Aquaculture. NTNU Ocean Science Week. Trondheim, Norway, May 5th, 2015.

Dissemination other

Nofima, 2015. Presentation of CtrlAQUA, Forskningsdagene, Sunndalsøra, September 2015
Nofima, 2015. Presentation of CtrlAQUA, Forskningsstorget, Oslo, September 2015



