





Norwegian Centre for Research-based Innovation

Nofima's facility in Sunndalsøra. Photo: Peter Breivik/TINGH ©Nofima. Front cover: Illustration of Neptun. (C) Aquafarm Equipment. Produksjon: MediaHuset AS, 6600 Sunndalsøra - post@mediahuset-as.no - 71 69 22 30

CtrlAQUA



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Introduction

Foreword (by Centre Director)

CtrlAQUA SFI came to its end 31st March 2023, after eight years with exiting and high-level industry relevant research, good discussions in the dedicated consortium, and having been a learning arena for more than 80 students and post-docs. In this final report we will summarise activities in the centre during these years. Further information and details can be found in the eight annual reports (2015 -2022), that are available on the CtrlAQUA website.

In addition to general information from the centre and some basic facts, this final report will also reflect on what CtrlAQUA has meant for the host institution and the partners. The partners agree that eight years provide time for long term discussions, building trust and network between partners. The regularity of meeting places, students, internal presentations and peer-reviewed publications have been important instruments for the consortium. For Nofima as the host institution, the centre has given us time and opportunity to build up our competence on the strategic important topic of further developing closed aquaculture technologies and securing health, welfare and performance in them. The

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Photo: Terje Aamodt, © Nofima

positive work environment and common interests within the consortium has triggered plans for future cooperation. Additionally, a supplementary agreement, signed by all partners will secure further collaboration on unpublished results and material.

The final report also delivers examples from research done in the centre and we will highlight some of the success stories that have been of particular interest for the industry. This research, more elaborately described in the annual reports and in close to 100 peer review publications, have been conducted by more than 50 researchers and technicians at the research infrastructures and around 80 master and doctoral students and post-docs.

On behalf of the CtrIAQUA leader group, I hope that you enjoy reading this final report, and please visit our CtrIAQUA website www.ctrIaqua.no for further information.

Sunndalsøra, 15th June 2023

Åsa Maria Espmark (Nofima senior researcher and CtrIAQUA SFI Centre Director)

Asa Maria Espmante

CtrIAQUA Final Report 2023

Foreword (by Head of Host institution)

Hosting the CtrlAQUA SFI has been a great journey for Nofima. CtrlAQUA has continuingly provided solutions for the aquaculture industry making an impact which is in the core of Nofimas mission as an applied research institute for food industries. Starting eight years ago with the main goal of making closed and semiclosed aquaculture systems an off-the-shelf technology this was an ambitious goal. Looking back at 2015 we can now all proudly acknowledge the work, resources and creativity provided by funders, industry partners and research partners. The mid-way evaluation of CtrlAQUA was excellent and results from the centre makes an impact on sustainable development of aquaculture. As a follow-up by the midway evaluation, Nofima has strategically invested to enable further knowledge for closed and semi-closed aquaculture systems to be developed. We have invested heavily in RAS infrastructure at the research stations in Sunndalsøra and in Tromsø. We have built a solid knowledge foundation with CtrlAQUA, and we continue to develop competence and capacity so that we can provide research-based innovations for sustainable aquaculture solutions also after CtrIAQUA.



Photo: Audun Iversen, © Nofima

During the eight years CtrlAQUA has had a very active board to guide the consortium in a constructive way. Nofima as the host institution are thankful for the major efforts of the CtrlAQUA board and all our collaborators and we look forward to developing sustainable aquaculture production solutions together.

Bergen, 15th June

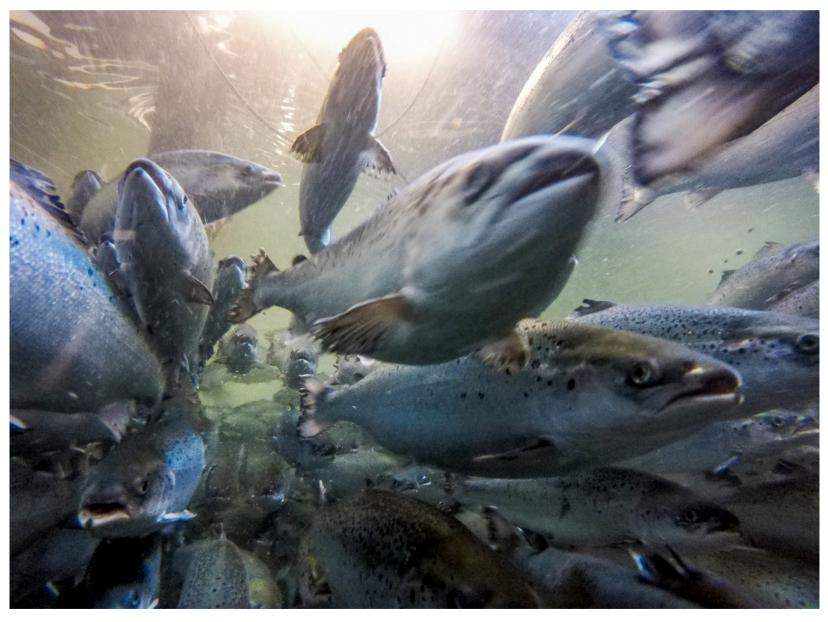
Bente E. Torstensen (Nofima Department director, Aquaculture)

Bente Morterer

Summary - CtrlAQUA

English:

CtrIAQUA SFI, Centre for Closed-containment Aquaculture, started when closed aquaculture systems were still quite new in Norway. Nofima built the first Norwegian R&D facility for recirculation aquaculture systems (RAS) in 2010, that has worked as the basis for the research done with RAS in CtrIAQUA. In the consortium we have also had some of the first producers and users of floating semi closed aquaculture systems (S-CCS), and we have therefore had access to research facilities in these systems. Closed aquaculture systems became a promising solution as lice and escapees prevented further growth in aquaculture industry. Production of postsmolt in closed systems also opened for the possibility to keep the smolt out of open net pens longer and reduce the risks of lice infestation and escapees. The CtrIAQUA



Post-smolt in recirculating aquaculture system. Photo: Terje Aamodt, © Nofima



Neda Gilannejad and Pradeep Lal from NORCE at the BENCHMARK sampling. Photo: Terje Aamodt, © Nofima

vision has been to contribute to a paradigm-shift in salmon aquaculture by providing novel solutions supporting further expansion of Norwegian aquaculture, thereby securing Norway's position as the worldleading supplier of aquaculture expertise, technology and sustainable seafood. The CtrlAQUA objective has been to develop technological and biological innovations to make closed-containment aquaculture systems 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.

To fulfil the objective, we gathered a consortium consisting of R&D partners with relevant scientific expertise, technology developers for RAS and S-CCS, fish farmers using RAS and S-CCS, and biotechnology developers. The consortium has had some changes during the centre life span. A few partners have left the centre, while others have been added as necessary replacements of those, and due to strategic changes after the mid-term evaluation, e.g., as we strengthened the consortium with water treatment

competence. The consortium has been stable throughout its lifetime with 21 partners, 7 from R&D and 14 from the industry.

The research within the centre has been divided between three scientific departments; 1) Department Technology & Environment; 2) Department Fish Production & Welfare; 3) **Department Preventive Fish** Health. The fourth Department of Training and Recruitment has taken care of the students in the centre. The annual plans have been created based on the centre description and inputs from the consortium. The research has been conducted in collaboration



The CtrlAQUA consortium at the kick-off meeting in 2015 at Nofima, Sunndalsøra. Photo: Terje Aamodt, © Nofima.

between R&D and industry partners, 17 doctoral students, 5 post-docs and 60 master students. All together we are very close to reaching the goal of 100 peer-review publications. The research will be further elaborated in the different Department sections in this report. A few examples include testing of different RAS smolt protocols in two life cycle experiments. Here we have tested performance, health and welfare of different protocols with light manipulation, fish size at sea transfer, early vs.

late inset of photoperiod, and long vs. short photoperiod. The tested protocols are based on industrial practices. Together with two associated projects (external projects to CtrlAQUA that have been approved to have rights and obligations to the centre), also focusing on industry driven protocols and input variables, we have contributed with knowledge that industry partners have reported that they use in practice. The semi-closed systems (S-CCS) were in the very early developmental

stage when CtrlAQUA started, and still today most of the systems are at the pilot stage. However, in the centre we have monitored health and welfare in the systems available in the centre, and we have contributed with hydrodynamic simulations to improve water inlet and distribution in the tanks. We have also tested UV with the aim to suggest how huge amount of water can be treated for different pathogens with UV technology. We have concluded that the tested systems work as

intended regarding health, welfare and performance. They have no lice, and the fish do not escape. However, as long as the systems are not 100% closed, some pathogens may enter and may cause harm to the fish. In addition to UV, CtrlAQUA has also contributed with knowledge regarding other disinfection regimes that have resulted in protocols that the industry has implemented. These include the use of ozone and peracetic acid, and a survey of the most commonly used disinfection protocols in the North American and Norwegian industries.

Since the use of closed aquaculture system is of international interest, it has been important for us to have international activities. Two of the R&D partners are from Sweden and USA, respectively, with expertise withing health, welfare and RAS technology. The Freshwater Institute located in West Virginia do research with salmon in RAS using freshwater throughout the whole life cycle. Their research on early sexual maturation, ozone and hydrodynamics have contributed much to the Norwegian industry as the production protocols and the use of freshwater have expanded. The use of RAS has also become more common in Sweden, and University of Gothenburg has contributed much to the results of skin and gut barrier function. The CtrIAQUA scientific advisory board (SAB) has contributed with external following up of

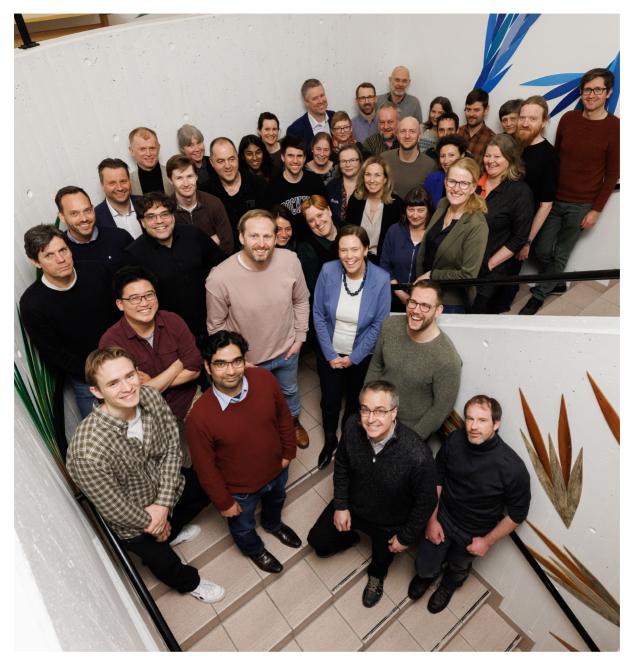
CtrlAQUA plans and results. The SAB almost entirely consist of international members representing the European Aquaculture Society, Danish Technical University and the University of Aberdeen. The only Norwegian representative represents the NGO Bellona.

Most partners in CtrlAQUA have given the feedback that apart from the scientific knowledge that have contributed to industrial practices, much of the added value from the centre has come from the consortium itself and the networking that the consortium has enabled. The partners report that a long-term project opens for discussing long term plans and possibilities for major changes. The atmosphere during the annual meetings and thematic meetings have been very informal and optimistic, and the willingness to share information. knowledge and experiences have been impressively high. This outstanding team spirit has led us to work towards continuing the collaboration after CtrlAQUA. We have had two meetings where we have discussed possible interesting topics and funding possibilities and many of the partners are already involved in new projects and applications.

Norwegian:

CtrIAQUA SFI, Senter for lukket akvakultur, startet da lukkede akvakultursystemer fortsatt var ganske nye i Norge. Nofima bygde det første norske FoUanlegget for resirkulering av akvakultursystemer (RAS) i 2010. Det meste av RAS forskningen innenfor CtrlAQUA har foregått i denne infrastrukturen. I konsortiet har vi i tillegg hatt noen av de første produsentene og brukerne av flytende semilukkede akvakultursystemer (S-CCS), og vi har derfor hatt tilgang til forskningsfasiliteter i disse systemene for å jobbe med CtrlAQUA målene for S-CCS.

Lukkede akvakultursystemer er lovende systemer ettersom lus og rømming hindret videre vekst i akvakulturnæringen. Produksjon av postsmolt i lukkede systemer åpnet også for muligheten til å holde laksen lengre borte fra åpne merder og muligheten for luspåslag og rømming. CtrlAQUA sin visjon har vært å delta i et paradigmeskifte innen lakseakvakultur ved å tilby nye løsninger som støtter videre vekst av norsk havbruk, og dermed sikre Norges posisjon som verdensledende leverandør av havbrukskompetanse, teknologi og bærekraftig sjømat. CtrlAQUA mål har vært å utvikle teknologiske og biologiske innovasjoner for å gjøre lukkede akvakultursystemer til en pålitelig og økonomisk levedyktig teknologi, for



The final CtrlAQUA consortium at the 2023 Annual Meeting in Sunndalsøra. Photo: Terje Aamodt, © Nofima.

bruk i strategiske deler av produksjonssyklusen for atlantisk laks, og dermed bidra betydelig til å løse utfordringene som begrenser den forutsatte veksten i akvakultur.

For å oppfylle målsettingen samlet vi et konsortium bestående av FoUpartnere med relevant vitenskapelig ekspertise, teknologileverandører av RAS og S-CCS, oppdrettere som bruker RAS og S-CCS, og bioteknologiselskap. Konsortiet har hatt noen endringer i løpet av senterets levetid, noen har sluttet, og noen har blitt ført til på grunn av nødvendige utskiftninger av partnere som har sluttet, og også på grunn av noen strategiske endringer etter midtveisevalueringen, f.eks. vi styrket konsortiet med vannbehandlingskompetanse. Konsortiet har vært stabilt med 21 partnere, 7 fra FoU og 14 fra industrien.

Forskningsaktivitetene i senteret har vært gjennomført i tre avdelinger; 1) Avdeling Teknologi & Miljø; 2) Avdeling Produksjon & Velferd; 3)

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Avdeling Forebyggende Fiskehelse. Den fjerde avdelingen for utdanning og rekrutering har hatt ansvar for studentene. Årsplanene er baserte på senterbeskrivelsen og innspill fra konsortiet. Forskningen er utført i samarbeid mellom FoU og industripartnere, 17 doktorgradsstudenter, 5 postdoktorer og 60 masterstudenter. Til sammen er vi veldig nær målet om 100 fagfellevurderingspublikasjoner. Forskningen i de ulike avdelingsdelene vil bli ytterligere utdypet i denne rapporten. Noen få eksempler inkluderer testing av forskjellige RAS-smoltprotokoller i to livssyklusforsøk. Her har vi testet ytelse, helse og velferd med bruk av forskjellige protokoller med lysmanipulasjon, fiskestørrelse ved utsett i sjøs, tidlig vs. sent innsett av fotoperiode, og lang vs. kort fotoperiode. De testede protokollene er basert på industriell praksis. Sammen med to assosierte prosjekter (definert som eksterne prosjekter til CtrlAQUA som er godkjent for å ha rettigheter og plikter overfor senteret), også med fokus på industrielle protokoller og innsatsfaktorer, har vi bidratt med kunnskap som industrien har rapportert at de bruker i praksis. De semi-lukkede systemene (S-CCS) var i et veldig tidlig utviklingsstadium da CtrlAQUA startet, og fortsatt i dag er de fleste systemene på pilotstadiet. Men i senteret har vi overvåket helse og velferd i systemene som er tilgjengelige i senteret, og vi har bidratt med

hydrodynamiske simuleringer for å forbedre vanninntak og -fordeling i systemene. Vi har også testet UV med det formål å foreslå hvordan man kan behandle store mengder for ulike patogener med UVteknologi. Vi har konkludert med at de testede systemene fungerer etter hensikten med hensyn til helse, velferd og ytelse. De har ingen lus, og fisken rømmer ikke. Men så lenge systemene ikke er 100 % lukkede, kan noen patogener komme inn og påvirke fisken negativt. I tillegg til UV har CtrlAQUA også bidratt med kunnskap om andre desinfeksjonsregimer som har resultert i protokoller som industrien har implementert. Disse inkluderer bruk av ozon og pereddiksyre, og en kartlegging av de mest brukte desinfeksjonsprotokollene i nordamerikansk og norsk industri.

Siden bruk av lukkede akvakultursystemer er av internasjonal interesse, har det vært viktig for oss å ha internasjonal aktivitet og innflytelse. To av FoUpartnerne er fra henholdsvis Sverige og USA, med kompetanse innen helse, velferd og RAS-teknologi. Freshwater Institute i West Virginia forsker på laks i RAS ved bruk av ferskvann i hele livssyklusen. Deres forskning på tidlig kjønnsmodning, ozon og hydrodynamikk har bidratt mye til norsk industri ettersom produksjonsprotokollene og bruken av ferskvann har utviklet seg. Også i Sverige har bruken av RAS blitt mer

vanlig, og partnerne fra Göteborgs universitet har bidratt mye til resultatene av skinn- og tarmbarrierefunksjon. CtrIAQUA scientific advisory board (SAB) har bidratt med ekstern oppfølging av CtrlAQUA planer og resultater. SAB er nesten utelukkende internasjonalt med medlemmer fra European Aquaculture Society, Danish Technical University og University of Aberdeen. Den eneste norske representanten representerer miljøorganisasjonen Bellona. De fleste partnere i CtrlAQUA har gitt tilbakemelding om at bortsett fra den vitenskapelige kunnskapen som har bidratt til industriell praksis, har mye av merverdien fra senteret kommet fra konsortiet selv og nettverksbyggingen som konsortiet har muliggjort. Partnerne rapporterer at et langsiktig prosjekt åpner for å diskutere langsiktige planer og muligheter for store endringer. Stemningen under årsmøtene og temamøtene har vært svært uformell og optimistisk, og viljen til å dele informasjon, kunnskap og erfaringer har vært imponerende høy. Denne enestående lagånden har ført til at vi jobber med måter å fortsette samarbeidet etter CtrlAQUA. Vi har hatt to møter hvor vi har diskutert mulige interessante temaer for videre samarbeid og finansieringsmuligheter. Vi har planer for fremtidige samarbeid og mange av partnerne er allerede involvert i nye prosjekter og søknader.

Vision and objectives

The Norwegian salmon industry and the government are aiming to increase the production in the years to come. However, this growth must be sustainable and not put the environment and fish health and welfare at risk. The ambitions for substantial increase in salmon production are big, but an increase depends on many environmental and social factors, including how we manage sea lice, pathogens and escapes, and how increased usage of land and sea areas needed for growth will affect other businesses and the public interests. Innovations in closed-containment aquaculture systems, where the salmon is separated from

the outside environment by a closed barrier, will be important for further development of aquaculture, since these technologies may be keys to solving many of the challenges. CtrlAQUA has been a centre for research-based innovation (SFI) that worked on such closed-containment systems. The main goal of CtrlAQUA SFI was 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

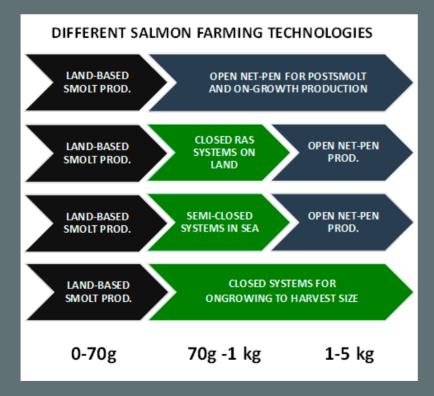


Figure 1. Different ways of producing Atlantic salmon: Closed-containment aquaculture systems.

solving the challenges limiting the envisioned growth in aquaculture".

Closed systems can be land-based where water is recycled (RAS), or sea-based, in which large floating tanks receive clean water from depth (S-CCS). In CtrIAQUA the research deals with both approaches. In the centre we have focused primarily on the most sensitive phases of the salmon production cycle, such as the first seawater phase, the socalled post-smolt stage (Figure 1). However, the research is also highly relevant for other strategies shown in the figure. The main innovation has been reliable and efficient production of robust postsmolts in closed and semiclosed systems on land and at sea. Thus, the industry can get a good realistic alternative or supplement to production technology with open cages. The centre has also contributed to better production control, fish health, welfare, and sustainability in closed-containment farms. We have done this through development of new and reliable sensors, methods for producing and recognizing robust fish, minimizing environmental impact through water treatment, reduce the risk of escape, and disease transmission to wild stocks

and optimizing tank/cage environment, amongst others. These innovations will be of value to the Norwegian society, since closed systems for strategic phases in salmon farming can contribute to the foreseen growth. From our perspective, we have achieved the goal of the centre. We have fulfilled innovations that have

improved the technological

and biological development

of closed closed-containment solutions (Table 1, p. 35). There are still developmental needs to complete the systems to become "off-theshelf" ready, especially for the semi-closed systems. Many of the challenges are on the regulatory side as the regulations prevent the systems to perform as intended. Also, there is need for further closing the systems with the development of treatment of inlet water and discharge to prevent pathogens and pollution. This will make the systems even more expensive than they are today, so to enable the full potential to prevent lice and escapees of these promising solutions, and thus to serve as sustainable solutions to the planned growth in aquaculture, appropriate regulations and conditions are required.

Neptun 4 is fitted with a water treatment system that includes UV as well as a sludge collection/ treatment system that will be retrofitted, making Neptun 4 a state-of-the-art sustainable semi-closed production facility. Photo: ©Aquafarm.



Basic facts about CtrlAQUA

Organisation

CtrIAQUA was organized (Figure 2) with a Board that oversees that obligations are fulfilled, and are responsible for finances, partnerships, and IPR issues, as well as ratifying annual research plans made by the leader group.

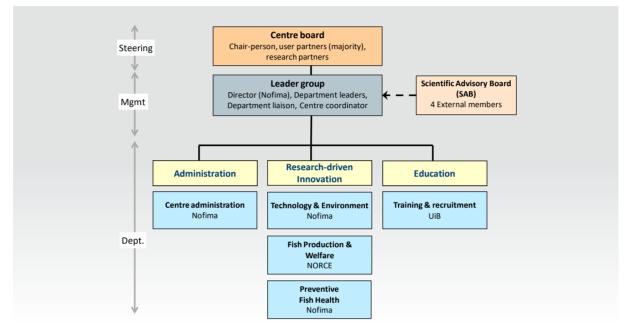


Figure 2. Organizational structure of CtrlAQUA

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Throughout the centre lifespan, the CtrlAQUA Board has had two physical meetings each year. The Board has consisted of the following elected members:

Name	Company	Role	Duration
Frode Mathisen	Grieg SeaFood	Chairperson	2015-2019
Harald Sveier	Lerøy SeaFood Group		2015-2017
Knut Måløy	Storvik Aqua		2015-2018
Siri Vike	PHARMAQ Analytiq		2015-2020
Tor Solberg	UNI Research		2015-2018
Mari Moren	Nofima		2015-2018
Harald Takle	Cermaq Group		2017-2023
Asgeir Knutsen	Krüger Kaldnes		2018-2020
Hans Kleivdal	NORCE		2018-2023
Hilde Toften	Nofima		2018-2023
Øyvind Oaland	Mowi		2019-2020
Trond Rosten	Mowi	Chairperson	2020-2023
Frederic Gaumet	Pure Salmon Technology		2020-2023
Rolf Hetlelid-Olsen	PHARMAQ		2020-2023

The CtrIAQUA Board 2023



Trond Rosten MOWI Chairperson of the CtrIAQUA Board



Harald Takle Cermaq Board Member



Hans Kleivdal NORCE Board Member



Hilde Toften Nofima Board Member and representing the host institution



Frederic Gaumet Krüger Kaldnes Board Member



Rolf Hetlelid-Olsen PHARMAQ Board Member



The first CtrlAQUA board gathered at the kick-off in Sunndalsøra in May 2015. Back left: Harald Sveier, Knut Måløy, Tor Solberg. Front left: Siri Vike, Frode Mathisen, Mari Moen. Photo: Terje Aamodt, ©Nofima

Each board member category (farming category, technology and biotechnology category, NORCE, Nofima) had a deputy. The Board members were suggested by an election committee consisting of three members and was led by the host institution. In addition, Kjersti Turid Fjalestad, the contact person for CtrIAQUA at the Research Council of Norway, was invited as observer at the Board meetings. The centre scientific work was organised through close collaboration between three departments: Dept. Technology & Environment, Dept. Fish Production & Welfare, and Dept. Preventive Fish Health, whereas student recruitment and management was managed in Dept.

Training & Recruitment. The Dept. of Liaison ensured smooth collaboration between departments and identified subprojects and user partners for projects. The leader group managed and lead CtrIAQUA, such as ensuring strategic planning and running of projects, recruitment of qualified personnel, and providing a good working environment and communication flow between partners. In CtrIAQUA there has been a focus on collaboration and knowledge transfer between the partners from the start. This collaboration has been done within the projects. and occurred between R&D partner scientists, scientists and user partners, and between user partners. The

extensive collaborations were facilitated by participation from all institutions in project workshops, thematic meetings, as well as joint experiments, sampling and analytical work. Frequent meetings were organized at Board level (each six months), centre level (annual meetings), leader group (every third week), and thematic or project level (as required). Furthermore, the Centre intranet had a news feed where centre participants have posted e.g. news, links to documents, research and results, pictures and videos. In addition to a formal news channel, the intranet has also been used as a social media, thus contributing to build the CtrlAQUA team spirit.

CtrlAQUA has had more than 50 researchers and technicians working within the centre the past eight years. Several of our senior researchers have also lead the research departments and our research projects:

- Department Technology & Environment (DTE): Bendik Fyhn Terjesen (until til 2017); Jelena Kolarevic
- Department Production and Welfare (DPW): Lars Ebbesson (until til 2017); Tom Ole Nilsen
- Department Preventive Fish Health (DPF): Sven Martin Jørgensen (until til 2016); Lill-Heidi Johansen

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The final CtrlAQUA leader group: rom left: Åsa Espmark, Sigurd Stefansson, Kasper Thøring Juul-Dam, Naouel Gharbi, Tom Ole Nilsen, Lill-Heidi Johansen and Jelena Kolarevic. Photo: Silje Katrine Robinson.



Jelena Kolarevic and Vasco Mota sampling. Photo: Terje Aamodt, © Nofima



Khurram Shahzad at the Nofima Centre for Recirculation in Aquaculture. Photo: Terje Aamodt, © Nofima



Sampling from PREVENTIVE-CARDIO experiment by Gerrit Timmerhaus, Carlo Lazado, Patrik Tang and Nikko Cabillon. Photo: Terje Aamodt, © Nofima



List of projects 2015 - 2023

Project leader	Project name	Duration	Dept. responsible
Andre Meriac (Nofima)	Water quality requirements in RAS and S-CCS (WATERQUAL)	2021 - 2022	DTE
Are Nylund (UiB)	Review of the microparasites that could represent a future problem for production of salmonids in closed or semi-closed containment systems (RISK)	2015	DPF
	Characterization of microparasites in closed and semi-closed containment systems (MICROPARASITES)	2015	DPF
	Microparasites in closed and semi-closed containment systems (MICROPARASITES)	2016 - 2022	DPF
Astrid Buran Holan (Nofima)	Particle and pathogen removal from intake water in semi-closed systems (INTAKE)	2015	DTE
	Particle tolerance in post-smolts reared in RAS (PARTICLE)	2016	DPW
	Efficiency tests of small-scale best solution from INTAKE1 (INTAKE)	2016	DTE
	Data analysis and evaluation from INTAKE 2016 and commercial scale implementation (INTAKE)	2017	DTE
Bendik Fyhn	CtrIAQUA SFI operation and administration (ADM)	2015 - 2017	All
Terjesen (Nofima)	Particle tolerance in post-smolts reared in RAS (PARTICLE)	2015	DPW
	Impact of alkalinity on postsmolt tolerance to CO2 in RAS (CO2RAS)	2016	DPW
	Health and performance in post-smolts when using novel production protocols (BENCHMARK)	2016	DPW
	Impacts of water quality on the performance and health of Atlantic postsmolts and biofilms in recirculating aquaculture systems (EXPO)	2017	All
Brian Vinci (FI)	Hydrodynamic challenges in big systems (HYDRO)	2019	DTE
Carlo Lazado (Nofima)	Disinfection strategies for improved biosecurity and fish health in recirculating aquaculture systems (DISINFECT)	2020	DPF
	Routine disinfection in RAS: fish health, microbiota, and pathogen control (DISINFECT)	2021	DPF
	Disinfection in RAS - fish health, microbiota, and pathogen control (DISINFECT)	2022	DPF
Christian Karlsen (Nofima)	Mucosal integrity, health and microbiota (BARRIER)	2016 - 2017	DPF

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CtrIAQUA

Project leader	Project name	Duration	Dept. responsible
	Skin function and health - changes in immune competence and susceptibility to diseases (BARRIER)	2018	DPF
	Barrier functions for better health (BARRIER)	2019	DPF
	Barrier protection and sensor monitoring of wounds (BARRIER)	2020 - 2021	DPF
	Barrier functions (BARRIER)	2022	DPF
Jelena Kolarevic (Nofima)	Machine vision for biomass in closed systems (BIOMASS)	2015	DTE
	Water quality in Flexibag semi-closed system for post-smolts (FLEXIBAG)	2015	DPW
	Modelling and water quality in Flexibag semi-closed system for post-smolts (FLEXIBAG)	2016	DPW
	AkvaVision for estimation of biomass and feed intake in closed systems (BIOMASS)	2016 - 2018	DTE
	AkvaVision for biomass estimation (BIOMASS)	2019	DTE
	Water quality requirements in RAS and S-CCS (WATERQUAL)	2019 - 2020	DTE
Khurram Shahzad (Nofima)	Hydrodynamic challenges in big systems (HYDRO)	2020 - 2022	DTE
Lars Ebbesson (UNI Research/ Norce)	Robustness evaluation parameters associated with biological requirements in closed systems (ROBUST)	2015	DPW
	Environmental and biological requirements & surveillance database for closed systems (DATABASE)	2015 -2016	DPW
	Innovative tools for assessment of post-smolt performance and development of the "Robust Post-Smolt Certificate" (ROBUST)	2016 - 2018	DPW
Lill-Heidi Johansen (Nofima)	Improved disease prevention, pathogen detection and immune prophylaxis in CCS (PREVENTIVE)	2018	DPF
	Improved health, disease prevention and pathogen detection in CCS (PREVENTIVE)	2019 - 2022	DPF
	Treatment strategies in S-CCS/RAS systems (TREAT)	2019	DPF
Naouel Gharbi (Norce)	Environmental conditions important for post- smolt performance and production of robust fish (ROBUST)	2020 - 2022	DPW
	Post-smolt performance and welfare in semi-closed containment (S-CCS) (RIGID S-CCS)	2021 - 2022	DPW
Pradeep Lal (Norce)	Optimization of production and rearing conditions (OPTIMIZE)	2021	DPW

Project leader	Project name	Duration	Dept. responsible
	Optimization of light production and rearing conditions (OPTIMIZE)	2022	DPW
Sigurd Handeland (Uni Research/ Norce)	Documentation of post smolt welfare and performance in large scale Preline semi-containment system (CCS) (PRELINE)	2015 - 2016	DPW
	Documentation of post-smolt welfare and performance in large scale Neptun 3 semi-closed containment system (NEPTUN3)	2016	DPW
	Documentation of post-smolt welfare, performance and water quality in commercial scale semi-closed containment systems (RIGID S-CCS)	2017 - 2018	DPW
	Data analysis and evaluation of results from INTAKE (INTAKE)	2018	DTE
	Performance in S-CCS (RIGID S-CCS)	2019 - 2020	DPW
Steven Summerfelt (FI)	Hydrodynamic challenges in huge tanks (1000+) (HYDRO)	2015 - 2018	DTE
	Assessment of photoperiod regime for freshwater- reared post-smolts (PHOTO)	2016	DPW
Sven Martin Jørgensen (Nofima)	Osmoregulatory barrier function in post-smolts reared in closed-containment systems (BARRIER)	2015	DPF
	Novel automatic monitoring system for improved diagnostics (POCNAD)	2016	DPF
	Improved disease prevention, pathogen detection and immune prophylaxis in CCS (PREVENTIVE)	2017	DPF
Tom Ole Nilsen (Uni Research/ Norce)	Temperature for optimal transfer of post-smolts from CCS (TRANSFER)	2016	DPW
	Carbon dioxide tolerance of Atlantic salmon post- smolts in RAS (CO2RAS)	2018	DPW
	Optimization of light quality and transfer conditions for post-smolts reared in closed systems (OPTIMIZE)	2017 - 2018	DPW
	Innovative tools for assessment of post-smolt robustness (ROBUST)	2019	DPW
	Optimization of production and rearing conditions (OPTIMIZE)	2019 - 2020	DPW
Trine Ytrestøyl (Nofima)	Health and performance in post-smolts when using novel production protocols (BENCHMARK)	2017 - 2022	DPW
	Comparison closed and semi-closed facilities (BENCHMARK)	2019 - 2020	DPW
Turid Synnøve Aas (Nofima)	Pre-project, new technologies for RAS sludge processing (REMOVAL)	2015	DTE



Project leader	Project name	Duration	Dept. responsible
Vasco Mota (Nofima)	Carbon dioxide tolerance of Atlantic salmon post- smolts in RAS (CO2RAS)	2017	DPW
	Impacts of water quality on the performance and health of Atlantic postsmolts and biofilms in recirculating aquaculture systems (EXPO)	2018	All
	Water treatment strategies for closed-containment aquaculture (INTAKE)	2020 - 2022	DTE
Øyvind Mikkelsen (NTNU)	Sensor protection & maintenance in closed systems (SENSOR)	2015 - 2023	DTE
Åsa Espmark (Nofima)	CtrIAQUA SFI operation and administration (ADM)	2017 - 2023	All

A Scientific Advisory Board (SAB) was appointed for CtrIAQUA, consisting of researchers and stakeholders with competencies in the fields of research in the centre. Important tasks of the SAB were to give advice during development of the annual plans and to evaluate the scientific work in the centre that annually ended up in a written report. The SAB consisted of the following members in 2022:

- Alistair Lane, European Aquaculture Society
- Anne Johanne Tank Dalsgaard, Danish Technical University (DTU)
- Kari Torp/Stefan Erbs, Bellona
- Samuel Martin, University of Aberdeen

Former members of SAB have been Yonathan Zohar - Univ. of Maryland, MD, US; Chris Secombes - Univ. of Aberdeen, Scotland and Julie Døvle Johansen - WWF Norway CtrlAQUA partners have repeatedly encouraged to networking activities as the interest and positive feedback from these activities have been very good. In addition to the annual meetings, the results have been disseminated through, in total 14 thematic meetings where eight have been physical and six have been held digitally. Also, all partners were invited to the Mid-term evaluation held at the Nofima research station in Sunndalsøra in 2019, and to the digital site visit from RCN, November 2020. The partners have been active during the production of the annual research plans. The planning of these always started with general discussions at the annual meetings, and were followed up by one-toone meetings between the centre director and individual partners.



Production hall RAS Nofima. Photo Terje Aamodt © Nofima

Research Partners and User Partners

R&D PARTNERS









Conservation Fund

USER PARTNERS

In addition to the below user partners, the following were also part of the original CtrIAQUA consortium: Firda Sjøfarmer (left the centre before the Consortium agreement was signed), Oslofjord Ressurspark AS (left in 2018) and Vard Aqua (left in 2019).























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Research

The Centre for researchbased innovation in closedcontainment aquaculture, CtrlAQUA, commenced operations in April 2015. The Research Council of Norway's objectives in running the SFIprogram 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 centre, formed the basis for the work in CtrlAQUA. Through close collaboration between user partners and the R&D institutions, the centre focused on closed-containment system innovations, such as new RAS process units, development and imple-menttation of prototypes and methods for improved fish

welfare and health, shown in Figure 3.

The work with the research annual plans was led by the leader group of CtrIAQUA, who used several sources of information to develop the plans, including: the SFI centre description, which was part of the proposal in 2014, the letters of intent by the user partners, meetings with the user partners, and input received from the partners during project, annual and thematic meetings (in total 14 thematic meetings). The Scientific Advisory Board (SAB) was appointed for CtrlAQUA, consisting of researchers and stakeholders with competencies in the fields of research in the centre. Important tasks of the SAB were to give advice during development of the annual plans and to evaluate the work in the centre that annually ended up in a written report.

of common projects and user-specific projects. Both types of projects contributed towards the main goal of the centre. Common projects were activities that benefit all partners in the centre, such as environmental requirements of salmonids in closed systems and optimal use of sensors, securing health and welfare, and hydrodynamic modelling. User-specific projects were defined as activities that also benefit the entire centre, but were particularly important for one user partner, or a group of user partners. From 2015, we also included associated projects, defined as: "A project can be termed an "Associated Project" to CtrlAQUA, and be entitled as such when applying for grants. The consortium behind this Associated Project must agree to share results with CtrlAQUA partners. The project owner of this Associated Project can participate at CtrlAQUA annual meetings, except when IPR-

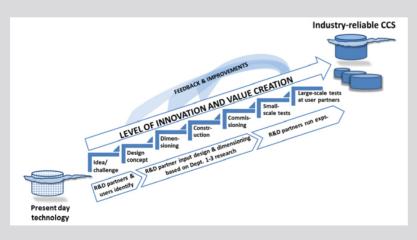
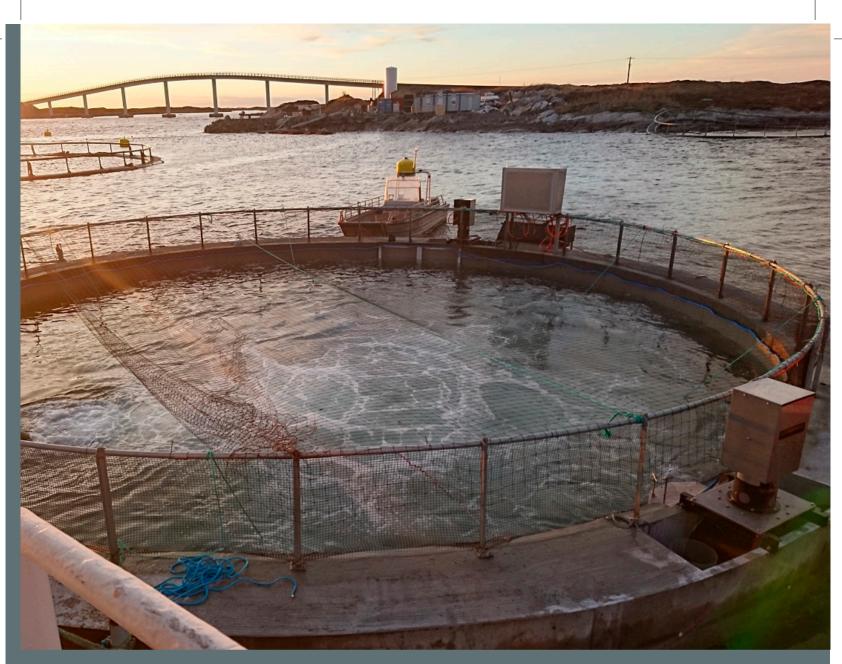


Figure 3. Innovation process in CtrIAQUA, from present day cage technology to establishment of industryreliable closed-containment systems, either in-sea closed tanks or land-based RAS. Exps. = experiments.



Pilot cage of 1.000 m3 floating semi-closed concrete sea cage at Gullklakken. Photo © Nekton

sensitive results are presented. CtrlAQUA partners will have no access rights or other IPR rights to results from the Associated Project, or vice versa, without written agreements similar to other third parties".

During preparation of the SFI centre description, several innovations were described and defined as innovation deliverables. These innovation deliverables are further linked to the departments and their specific research tasks. In the annual plan, each project was linked to one or more Innovation deliverables, and this was an important tool during discussions of the research plans. Innovations were also defined when user partners implement CtrIAQUA results into their businesses as improved routines or operations.

After the mid-way evaluation of CtrIAQUA in 2019, three new focus areas were implemented that were not part of the original centre description but became relevant after the kick-off. These were issues with hydrogen sulfide that have caused many incidents of mass mortalities in RAS facilities using salinity, nephrocalcinosis and early sexual maturation that are two production related issues mainly in RAS, but also in flow-through systems. These three subjects were important parts of CtrlAQUA since 2019 and have resulted in main activities, implementations in the industry, PhD students and associated projects.

Research achievements

Department Preventive Fish Health

The main objectives in **Department Preventive Fish** Health were inventions to prevent, detect and control diseases in closed and semiclosed containment systems. This may be obtained by different measures, such as strengthening fish robustness and disease resistance, improving pathogen control and handling of disease outbreaks, securing stable conditions in the production systems to avoid potential risks to fish health and welfare, and by developing new or refined vaccines and vaccination protocols for pathogens representing a special threat in these systems.

The use of S-CCS in the marine production phase of salmon was expected to prevent or reduce the influx of micro- and macroparasites if stocking pathogen-free smolt and controlling the intake water. However, the introduction of microparasites which can spread by direct transmission, could pose a major threat for the future success of these systems. It was hypothesised that S-CCS could reduce the number of farmed salmon populations that will become infected by microparasites, and they could also reduce the importance of farmed populations as reservoirs for microparasites if the effluent

water from these systems was treated. On the other hand, production in these systems may potentially offer new fish health challenges. Novel pathogens and new impacts of known pathogens could be a risk as higher population densities and slower exchange of water is expected in these systems. An extensive review of the microparasites that could potentially represent a future problem for production of salmonids in closed or semiclosed containment systems was made in the early phase of CtrlAQUA SFI. A main objective was thereafter to identify and characterize the most important known and emerging microparasites in S-CCS by developing methods and technology for fast and specific identification of microparasites and mapping the diversity, prevalence, load and transmission routes. Different S-CCS at CtrlAQUA user partners from north to southwest of Norway, have been compared with open net pen systems and land-based RAS smolt production facilities. The studies have been performed in several production cycles in the different systems. The hypothesis was that production of salmon in S-CCS does not have a negative effect on the prevalence and loads of microparasites compared to open production systems (sea

cages). Based on the findings in the studies which have run throughout the whole 8-year project period, we conclude that S-CCS will not affect the diversity, prevalence and load of parasites negatively compared to open production systems and S-CCS does not give higher mortalities compared to open systems. S-CCS may prevent the exposure of salmon from some marine microparasites. Atlantic salmon in S-CCS are to a certain degree protected from infection with salmon lice, but there are no significant differences in development of prevalence and densities of other microparasites in S-CCS compared to nearby open cages. The risk of introducing marine microparasites into S-CCS also seems to be related to the depth of the intake water. The water intake of S-CCS should, if possible, be below the planktonic layer and preferably below 30 meters but with the reservation that this could lead to introduction of other fish pathogenic bacteria present in the water column. On the other hand, several other known microparasites, like ISAV, IPNV, PRV, SPGV and Ca. B. cysticola, are frequently found in smolt production sites and may follow the smolt from freshwater into rearing systems at sea. Future production should focus

CtrIAQUA Final Report 2023



Cermaqs S-CCS facility Certus has played a key part in CtrlAQUA. Photo: © Cermaq

on high smolt quality, e.g., smolt free of microparasites, for postsmolt production in S-CCS. Research is still needed for optimalization of the different S-CCS for preventing introduction of microparasites.

Novel genotyping tools for selected pathogens have been developed in CtrIAQUA. One example is the bacterium *Candidatus* Branchiomonas cysticola which is considered the primary cause of epitheliocystis in farmed salmonids. So far, no genetic characterization has been done to describe potential differences associated with geographical origins, host species or reservoirs, primarily due to lack of cultivation systems for this intracellular bacterium, which complicates the sequencing of its genome. Reconstructing the evolutionary relationship of this bacterium compared to its closest related species has revealed its unique characteristics, and the genetic divergence observed has led to a proposal of a novel bacterial family to be named Branchiomonaceae. Results show that it is a small selection of highly similar strains/ variants of the bacterium that is circulating in farmed and wild salmon in Norway. A genotyping scheme has been developed which enables the possibility to attribute observable traits of the variants that were identified. Whether

the few genetic differences observed affect virulence remains uncertain and will rely on clinical field observations.

Pathogen surveillance is an important biosecurity measure in aquaculture. However, the current analysis turnaround time is a bottleneck for efficient management practice to prevent and minimize risk of disease. A prototype system to perform automatic sample treatment, purification of DNA/RNA and amplification and detection of pathogens in water samples, named FORDETECT, has been under development throughout the CtrlAQUA lifespan by the University of SouthEast Norway (USN). An international patent

application on the system is pending. Tests performed on automatic DNA purifications of water extracts were shown successful. USN, together with a commercial partner, will continue to standardize and test the FORDETECT system directed towards salmon farming.

Novel tools developed in the project period also include the multigene assay. The method makes it possible to evaluate smoltification - and immune status in groups of fish at the critical stage before and after seawater transfer, by simultaneous assessment of several genes from the same sample. The assay has been successfully tested in largescale salmon production trials in CtrIAQUA studying different production protocols and their potential effect of fish robustness and immune status and is now used by the industry.

Due to the finding in the pathogen surveillance and screening studies, that no novel pathogens in S-CCS was detected, focus related to vaccines shifted towards developing vaccination strategies adapted to postsmolt production in S-CCS and optimization of existing vaccines. For winter ulcer disease (causative agent Moritella viscosa), although commercial vaccines are available, severe and numerous outbreaks of ulcerative disease in farmed Atlantic salmon are an impeding factor for sustainable growth within the industry. To understand how basic mechanisms in host-pathogen interactions and vaccine-associated



Post-smolt. Photo: Terje Aamodt, © Nofima

protection of the skin can influence development and outcome of skin infections, we have investigated effects of intraperitoneal (ip) vaccination and enduring immune modulations and responses to infection in the skin of Atlantic salmon. Vaccination resulted in protection to both M. viscosa induced mortality and skin ulceration. However, infection is still established regardless of vaccination. Image analysis brought novel insight into the infestation strategy of the bacterium: M. viscosa initiate infection by colonizing the scale surface and cause scale degradation. Tissue responses to infection include influx of blood and immune cells between the outer surface of the scale and the outermost skin layer, epidermis, and thickening of the epidermis. Gene analysis suggests that vaccination affects different skin layers differently, but that also skin respond immunologically to ip vaccination. Furthermore, vaccinated and unvaccinated fish seem to respond to infection in different skin layers, and mediators of skin immunity are primed in the epidermis in vaccinated fish. The work has contributed to the improvement of the vaccine against winter ulcer disease.

In the project we worked to understand the mechanisms that enhance fish robustness with regards to host-microbial relationships, and intrinsic and extrinsic barrier functions. Being in direct contact with the water, the fish skin has important roles in defence and protection against pathogens. To reduce production costs in closed systems, high fish density is advantageous, but only if this does not adversely affect welfare and health. Our findings show that the skin of post-smolts from closed systems reflects both fish density and rearing technology, almost as a living sensor, and both the cell numbers, morphology and gene expression in skin reflect the environment. While traditional indices such as growth rate or blood physiology offer little information about the effect of treatments, skin gene expression clearly indicated when water flow is too low. These findings can be used to monitor fish and conditions and overall improve how we farm salmon in closed systems in the future.

Comparing freshwater smolts to post-smolts after 1 and 4 months in seawater and using two different production systems, open net pens and S-CCS, showed gradual morphological development in skin thickness and mucus cell numbers with growth and time post seawater transfer Gene expressions suggested similar processes. We also observed a delayed recovery of immune functions related to gradually enforced protection against pathogens. Overall, the period after seawater transfer could be considered as a barrier recovery phase where the salmon builds resilience and robustness for further growth. This could be an important

argument for using rearing facilities that reduce encounter with fish pathogens during the first months in the sea. That the immune system of smolts weakens in the period immediately after transfer to sea and that the fish use a few months were they gradually recover, will make them more susceptible to infectious diseases in this period.

Salmon feed ingredients have changed dramatically over the last years, from marine to plant-based ingredients. How different feed ingredients affect the skin is not fully known. From the parr stage, contrasting diets composed of either marine - or plant ingredients were used to assess how they affect the skin during the last stage of smoltification and the first period in seawater. In smoltified fish, growth in the marine diet group was significantly faster. The deeper layer of the skin was thinner in the plant fed group and the number of genes regulated in skin was higher in this group when transferred to seawater. Many of the genes were related to the immune system. However, feed-related differences in the skin were smaller compared to environmental changes, i.e., the effects of transfer from freshto seawater. In the intestine, gene expression suggests that a marine diet stimulates blood flow, development and immunity. However, histological studies showed little dietary impacts on these tissues. Instead, intestinal structures were affected by the faecal

consistency score that is linked to appetite or time since last feeding. This observation is in parallel with several blood parameters. Blood parameters related to stress (cortisol) or osmoregulation showed no difference between dietary groups after seawater transfer. From the study we cannot conclude that plant-based feed poses negative functional effects on skin.

Knowing the healing progression of different wound types when injuries occur can be used to estimate a time frame for healing, e.g. if the tissue recovers and restore or if further handling increases the risk of adding further damage to the skin. When assessing skin structure characteristics, we revealed effects and wound healing progression of different mechanically induced skin wounds. Wound healing progression depends on the severity of damage. After 5 weeks at 10 °C, scale loss areas were in the final remodelling stage, superficial wounds were closed but only starting to develop scales, while deep wounds were still in inflammation with active tissue formation. Furthermore, samples from fish reared with different light production protocols in RAS were used to investigate functional and morphological characteristics of the skin and intestine. No effect of light treatment (12:12 vs. 24h light) was observed on the barrier and transport functions of the skin at 100 gr. The intestinal barrier and transporting functions in the

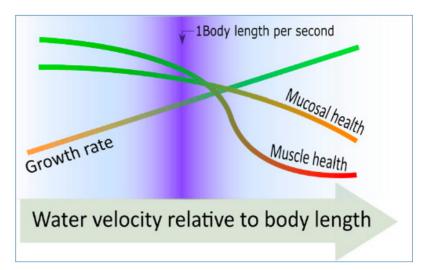


Figure 4. Simplified overview of water velocity effects on postsmolts in RAS.

12:12 group resembled changes previously observed during natural smoltification. Later, at ca 300 gr, and including both the variables constant light and early and late winter signalling, differences in skin morphometrics in neither fresh nor brackish water were seen.

Optimal water velocities in tanks and CCS are important for achieving improved cardiac health, increased robustness and disease resistance in Atlantic salmon. Upon start of the project, there was little knowledge regarding optimal water velocities for postsmolts during the first period in seawater and onwards. One of the objectives in the department was therefore to identify water velocity thresholds for optimal growth and robustness of both smolts and post-smolts in CCS and RAS. In the first big scale trial at a commercial smolt production site, increased water speeds of 0.6 body length (BL) per second ("Low speed") and 1.0 BL per second

("High speed") were tested. Both weight and length increased significantly in the high-speed group during the experiment showing that higher water speeds have the potential to increase the growth rate of large smolts. In a dedicated and controlled experiment, we investigated the effects of low to very high water-velocities on A. salmon smolts in RAS to gain further insights into optimal rearing conditions. We evaluated growth, muscle development, schooling behaviour, welfare scores and immunological and stress responses of fish in four different velocity groups (0.5, 1.0, 1.8 and 2.5 BL/ second (s)) over three months. Findings included that fish in higher velocities form denser schools and certain fin damages were slightly more frequent. However, the overall external welfare scores remained favourable in all groups. Significant increased mean body weights correlated to the applied velocities, with the largest growth found in

the 2.5 BL/s group, and this was linked to increased muscle growth. Plasma cortisol, used to validate stress level, was significantly elevated for the 2.5 BL/s group during the first week of the trial. However, these differences disappeared to the end of the trial. In conclusion, we found overall beneficial effects of elevated water velocities, but some results indicated possible negative effects for fish in the highest velocity groups, indicating an optimum velocity slightly over 1 BL/s for smolts in RAS. Adaptations to large commercial systems need to be developed and tested.

Rearing fish in a highly controlled environment requires that water quality is at an optimum and that a system is in place to prevent potential disease outbreaks. In S-CCS intake water treatment is used to create a barrier that prevents pathogens from entering the facilities. Unlike land-based RAS, the lack of water reuse and the resulting large flows in S-CCS $(\geq 500 / \text{min})$ pose a significant challenge for finding safe and financially viable intake water treatment strategies. We reviewed experimental data and literature for relevant filtration and disinfection technologies and conducted an informal survey with technology users and suppliers to discuss opportunities. A combination of microfiltration and UV treatment remains the most promising and cost-effective approach to treat intake water in S-CCS.

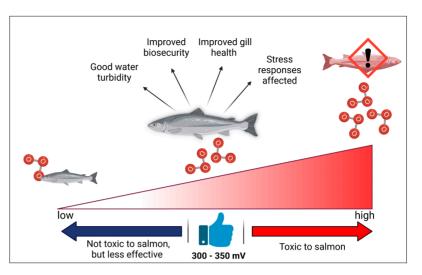
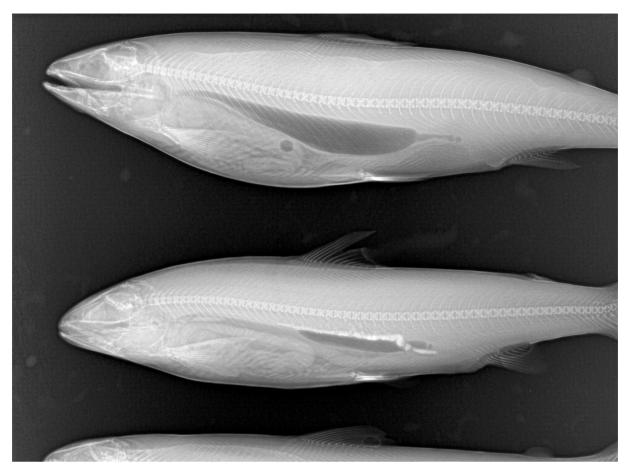


Figure 5. Ozone and its effect on salmon.

Other novel technologies were also identified as potential applications in intake water treatment. Due to lower flow rates in RAS, membrane filtration could be an attractive option to prevent pathogens from entering S-CCS. Optimizing energy efficiency of treatment technologies and guantifying "acceptable risk" in operation are keys for developing a sustainable concept for intake water treatment in the future. We also mapped the disinfection strategies used in RAS facilities in Norway at user-partners, and surveys showed several similarities. but also dissimilarities between the strategies in different facilities. Majority of the surveyed facilities had an inhouse disinfection protocol however the disinfection efficiency of the strategies had not been verified experimentally. Peroxidebased disinfectants, (mainly peracetic acid) were the most common used chemical disinfectants, conforming to the approved disinfectants

for aquaculture use by the Norwegian Food Safety Authority. Based on the user partner-surveys, we evaluated chemical disinfectants and pH manipulation in MBBRs. All protocols effectively disinfected the biofilter, however, recovery time varied. For instance, systems disinfected with chlorine recovered faster than those disinfected with peracetic acid (PAA) and a guaternary compound. The results contributed to improve and shorten the disinfection processes in the industry.

Ozone treatment remains as one of the most potent water disinfection methods in RAS. We explored the impacts of chemical-mediated strategies on system performance, water quality, biosecurity and fish health and documented the impacts of ozonation by-products, total residual oxidants (TRO`s), after ozonation of brackish water, on fish and welfare. The highest levels of TRO tested significantly affected the



The upper fish is healthy while nephrocalcinosis can be seen in the center of the lower one. Photo: Svein Alexandersen/Pharmaq Analytiq.

gill health of salmon that eventually resulted in mortality. Conclusion was that TRO levels of ca 300-350 millivolt (mV) represents a threshold for Atlantic salmon in brackish water RAS systems related to health and welfare. The impacts of PAA and ozone application as routine water disinfection protocols on salmon smolts in brackish water were also evaluated. Fish were first exposed to periodic application of low dose PAA without any negative effects. However, when fish were prompted with a secondary stressor (acute confinement test), the group treated with PAA tended to recover relatively slower (as shown by development

of cortisol and glucose levels). Further, the impacts of continuous ozonation on the health and welfare were explored, using the ozone concentration 320-350 mV, earlier identified to be safe for the fish. No mortality was registered, and fish external welfare scores were superior. Ozone may also have other properties than water disinfectant. To study if water ozonation can reduce or remove waterborne sex hormones in RAS that may give early maturation, water samples from RAS with or without ozonation were analysed for concentrations of the sex hormones testosterone, 11-ketotestosterone (11-KT),



Nephrocalcinosis can be seen as the white lines on the left side of the fish: Photo: Inger Lise Breivik/Bremnes Seashore.

estradiol (E2), and cortisol. Even though 11-KT and E2 were lower in ozonated RAS, maturation was still high in both test groups. Thus, other yet not identified environmental factors seems to have a strong impact on maturation.

In the last 2-3 years of the project, we worked to gain insight into the causal factors behind nephrocalcinosis (NC; calcification of kidney) and the physiological mechanisms associated with this condition that had increased in severity in later years. A literature review and a survey within the consortium was performed to get information about possible causes of NC. There were no clear conclusions about cases of NC, and it is likely caused by several factors. Some general results were that it is first seen in very small fish (< 15 grams), it has increased in severity the last years, it is more common in brackish water, it occurs both in RAS and flow through systems and it may be related to mineral composition in feed and choice of buffer. Finally, NC is not solely a CO₂ problem. In the search in grey and white literature, CO₂ had been raised as a causative factor, together with sudden changes in water quality parameters, imbalance in minerals, and the combination with high densities and low water exchange.

To gain a better understanding of the physiological perturbations associated with NC we have also addressed normal physiology, as remarkably little knowledge exists about changes in kidney functions in smolts and early post-smolt during transition from freshwater to seawater. A preparatory increase in kidney Na, K-ATPase (Nka) enzyme activity takes place as part of the smoltification process. This occurs significantly later than the classical increase in gill Nka and suggest that Nka activity in the kidney requires more time to reach peak activity levels. Thus, there may be a risk of osmoregulatory dysfunction in smolts or post-smolts if they are prematurely transferred to higher salinities, which in turn may apply to other key transporters and therefore contribute to precipitation of salts in the kidney. Such osmoregulatory dysfunction may in turn affect the prevalence of NC, particularly since water quality may vary with respect to ion composition and high salt content of feed in modern RAS.

As no non-invasive methods existed to diagnose the condition, the suitability of radiography (X-ray) to identify NC at different developmental stages and at different life stages of the salmon. The benefits of using radiography for diagnosing NC are that it is non-invasive and easily performed on live fish, it is quick and can be done on large numbers of fish at the fish production sites using portable equipment. This also enable to follow the development in fish with a known degree of NC throughout production and study their survival,

performance, and growth at sea to learn more about the consequences this diagnosis has for the fish. Several hundred Atlantic salmon from 9-1000 g, have been radiographed and examined for NC, many while alive. Other methods included for comparison were histology, the main diagnostic tool used today, and macroscopical scoring. Comparisons show that there is a correlation between histological and macroscopical scoring, and radiography. We have seen that accuracy of radiographic scoring depends on image quality, fish size, experience, and the degree of NC. Image quality improves with increasing fish size and increasing degree of NC and larger mineral deposits increases visibility on the radiograph. Stationary radiography (mammography) equipment especially designed to detect small calcifications in soft tissue, gives better images than portable x-ray systems, but smaller changes can be recognized with increasing experience by the user. Tests show that with the portable system even lowgrade NC on 40 gr fish can be detected. There is a great need for a common scoring system to get comparable scores for the understanding of the cause and effect of NC. Blood was analysed for several compounds, and it revealed that primarily the Magnesium levels were out of normal range in the NC affected fish.

Department of Fish Production and Welfare

Production of smolts and postsmolts in RAS facilities provide a great opportunity to control the production environmental parameters. Hence, the main priority within Department of Fish Production and Welfare is to provide knowledge and innovations to determine environmental and biological requirements of Atlantic salmon in RAS and semi closed systems (S-CCS).

During the CtrlAQUA centre lifetime, we have benchmarked production protocols in RAS in order to provide recommendations for best practices. The optimal strategy for rearing large post-smolts in land-based recirculating aquaculture systems, with respect to salinity and water velocity was assessed. In this long-term study, post-smolts from 70 g up to 800 g were reared in three separate RAS at different salinities of 12, 22 and 32‰ and subjected to

moderate (~1 bl s-1) or low (~0.3bl s-1) water velocity. Results suggest that salinity isotonic to the fish (12‰) and moderate training (~1 bl s-1) has a positive effect on postsmolt growth, feed efficiency, welfare, and survival in RAS. At 250 and 800 g, all treatments handled sea water transfer, while at 450g handling and transfer caused high mortality in several treatments. The present study in combination with previous studies suggest that the optimum velocity for salmon post-smolts is slightly above 1 body length per second. Further, using water with salinity at around 12‰ may therefore be a production strategy for large post-smolts in RAS, provided that post-smolts can handle the subsequent transfer to open sea cages. Hence, we followed up with two long-term benchmark studies to verify best practices with respect to salinity, light regime and

transfer time of smolts and post-smolt from RAS to sea cages.

It was important to clarify if a prolonged land-phase in RAS and transfer at a larger size would improve seawater performance of Atlantic salmon. In the first study, seawater survival and growth performance was compared in salmon given a winter signal that stimulate smoltification in salmon or just using 24 hours light during the entire RAS period using both freshwater and in brackish water. Fish were transferred to sea cages at three different sizes of 100 g, 200 g and 600 g. The overall results suggest that to avoid negative effects on growth and survival after sea transfer, it is considered safe to keep salmon on land until they are 200 g and then transfer them to sea. Using 12 ppt gave better growth in RAS compared to using freshwater,

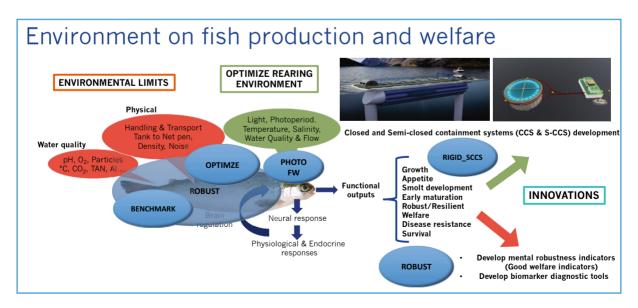


Figure 6. A schematic overview of the Department of Fish Production and Welfare.





High activity when researchers, students, and technicians from Norce and Nofima are sampling in the project BENCHMARK at Nofima in Sunndalsøra. Photo: Terje Aamodt, © Nofima.

particularly for fish on 24h light. Thus, using 12 ppt in RAS may be beneficial when fish are not given a winter signal to induce smoltification. Moreover, a winter signal, stimulating smoltification in RAS may not be necessary for fish transferred at 200 and 600 g as fish above 200 g produced with 24h light in RAS were largest at slaughter for both transfer sizes. However, it is noteworthy that the larger smolt groups did not perform as well as 100 g controls produced with winter signal

and transferred to sea cages in August. Transfer at 600 g can, despite lower growth in seawater, still be beneficial due to reduced time in seawater in areas heavily affected with salmon lice. These findings raised the question of whether delaying and prolonging the winter signal in RAS could improve seawater performance of Atlantic salmon transferred to seawater at a larger size. In the second study, smolts and post-smolts were transferred to sea cages at three different sizes of 100 g, 200 g and 600

g. The results suggest that a traditional winter signal given when the fish are 40 grams in RAS improves growth performance in seawater but reduces growth rate in RAS. This protocol may increase the risk of inducing early maturation in males if the fish are kept in freshwater RAS up to around 800 g before transfer to seawater. Thus, an early winter signal should be used with caution if the smolts are to be kept in RAS and transferred to sea at a large size. Fish transferred in

September at the smallest size had the best growth performance, survival, and final bodyweight. Hence, the better performance of fish transferred earlier in the fall at a smaller size compared to larger fish transferred later in the fall is in line with the results from the first study described in the text above. Thus, it seems that transfer of large fish in late fall is not a good strategy for optimal seawater performance.

It is not just the changes in daylength and salinity size at transfer that may impact fish performance in RAS. Other factors such as the type of light and water quality is also important. With the emerging new LED light technologies, both the light intensity and spectral composition can easily be manipulated. How this may affect growth, maturation, welfare and saltwater tolerance of salmon post-smolts in RAS is therefore important. We found that "sun-like" full spectrum LED light was not beneficial compared to regular white LED light as red light at low intensity did not affect performance, welfare, or development of salt tolerance in salmon post-smolts. Increasing light intensity at the bottom of the tanks did not have any benefits for growth performance while untreated RAS water creates a steep light gradient in the tank. Surface illumination in deep commercial tanks may not be sufficient to maintain light intensities above thresholds relevant for vision or maturation, suggesting that light

intensity is more important than spectral composition for Atlantic salmon postsmolt performance in RAS. Nevertheless, if light intensity is a limiting factor for salmon production in deep tanks, surface light can be supplemented by underwater light for more local effect and further on by improving water clarity of RAS water using advanced water treatment (skimmers, ozone, etc.). High carbon dioxide (CO₂) levels in water is expensive to remove and may have negative impact on the fish. We therefore determined the long-term effect of five different CO₂ levels, from 5 to CO₂ concentrations in 12 ppt salinity RAS up give the growth performance, health, or welfare of Atlantic salmon post-smolts. The growth in Atlantic salmon post-smolts is negatively linear-related to CO₂ exposure, indicating that the growth penalty of CO_{2} starts at lower concentrations than previously reported (<12 mg/L). An approximate 10% of growth reduction was observed for every 10 mg/L increase in CO₂, over the range of CO₂ concentrations studied (5-40 mg/L). Thus, it is recommended to avoid water CO_2 concentrations >12 mg/L in RAS as it might affect further growth in the sea phase. Development of early puberty in large smolts and post-smolts when reared under continuous light and high temperatures has been important questions that needed to be resolved during the centre time. Based on our findings in the literature

we have identified some key factors that may reduce the risk of early puberty. To avoid maturation, it is recommended to avoid temperature increase above 13°C in RAS, especially for the larger post-smolt sizes (100 and 250 g). Providing an artificial winter photo signal to induce smoltification (as opposed to other means) could have a preventative effect on early male maturation during the post-smolt phase. To minimize risk of maturation under 24h light regime, keep temperature < 12°C.

Commercial feasibility of farming post-smolt Atlantic salmon in closed sea systems relies on maximizing fish density, minimizing specific water flow, and determining biological limits for salmon in these environments. To this end, post-smolts reared at five different (25, 50, 75, 100 and 125 kg m-3) in fullstrength seawater shows that increased stocking density had a negative effect on growth and feed utilization, and from the 100 kg m-3 to 125 kg m-3 treatment a 42 % decrease in post-smolt growth rate was observed, suggesting that max rearing density should not exceed 75 kg m-3. In commercial systems operating with large water volumes this will be a substantial energy cost. Even small reductions in the volume of water that needs to be pumped may have a significant effect on reducing costs. Determining the massspecific water flow (SWF) required by post-smolts will largely influence the design



Erik Heimdal, Master student (NORCE), joining the Benchmark II sampling in Sundalsøra. Investigating smolt physiology and performance: small and large smolt. Photo: Terje Aamodt, © Nofima.

and dimensioning of closedcontainment systems in the sea. Overall, it appears that post-smolts reared at densities lower than 75 kg m-3 can adapt to reduced flow down to 0.2 L kg fish-1 min-1. However, it is recommended that without any in-tank water treatment, specific water flow should be maintained above 0.3 L kg fish-1 min-1 as physiological regulatory responses may be costly for the fish and have negative impact on growth and welfare.

In the centre lifetime, CtrIAQUA has documented and compared growth, performance, welfare, mortality and lice infection in Atlantic salmon raised in large scale semi-closed system (S-CCS: Preline, Neptun and Certus). In addition, selected production

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data from six generations of salmon produced in S-CCS Preline have been used to compare growth and performance in fish raised in S-CCS and open sea cages. The benchmark study was carried out in two phases. Phase one used post-smolts from 100 g to 800 g in seawater, and fish in S-CCS were compared with a reference group from an open sea cage. The second grow-out phase used salmon from 800 g to 5000 g in open sea cages, where fish previously reared in S-CCS were compared with fish from a reference group. The findings from the benchmark analyses showed a significantly lower infestation of sea lice in Preline fish during the postsmolt phase. Furthermore, in the grow-out phase the Preline group showed higher weight gain and final weight compared to the reference group in open pen. Salmon raised in Preline showed significantly higher survival compared to the reference group, indicating increased robustness in fish raised in S-CCS when transferred to open net pens in sea. In line with the Preline findings, data from the Certus concept show 14 % improved post smolt growth and minimal with sea lice when compared to reference fish in open pen. Moreover, fish from S-CCS showed lower cortisol and plasma ion levels at baseline. giving a stronger response to acute stress challenge than fish from reference, suggesting a lower and more adaptive stress response in the S-CCS than in the reference open pen. These results provide a foundation for a linear economic model that will be used to analyze the effect of implementing a new S-CCS post-smolt production strategy against a conventional open net pen production line. Future large scale benchmark studies should provide more comprehensive conclusion of the potential of S-CCS strategies in the future.

Department Technology & Environment

The main objective of Department Technology & Environment was to facilitate innovation of closed-containment system technology, water treatment processes, and sensors, to achieve a high level of production control. During lifetime of CtrIAQUA larger culture tanks were developed to reduce capital and labour costs per ton of fish produced in both floating and landbased closed-containment systems. In RAS facilities on land circular and octagonal tanks were mostly built with single or dual drains with volumes approaching 1000 m³. However, upscaling of the tanks presented many hydrodynamic challenges. Larger tanks made it harder to simultaneously achieve rapid solid removal (tanks self-cleaning), ideal swimming speeds for fish and homogenous water quality across whole tank volume. The results of a survey from the start of the centre showed that the mean culture tank volume ranged from 500 to 1300 m³ with 14.5 to 20 m diameter and depth of 3.5 to 4.5 m (diameter to depth ratio of 3.6:1 to 5.5:1). Water flow through each large culture tank ranged from 3 to 19 m³ and the mean hydraulic retention time (HRT) of water in the tank between 35 and 170 minutes; in tanks built after 2013 HRT ranged between 34.8 and 52.5 min while in the tanks built before that it was between 67 and 170 min. This indicated the importance of

efficient flushing of metabolites from the water to keep good water quality and potentially support increased feed loads in the tanks.

Computational Fluid Dynamics (CFD) modelling approaches were used to study hydrodynamic properties of large tanks indicating that the large tanks are more turbulent than smaller units, which can be a problem since water velocity may be too high or too low in certain parts of the tanks, also creating dead zones that are not turned over sufficiently. We showed that by doing relatively small construction changes, such as change of design or orientation of the water inlet pipe in the tank, these turbulent parts may be reduced.

The empirical measurements in large commercial tanks showed that presence of fish can decrease water velocity in tanks by 25% and that water quality (such as oxygen saturation in the tank) exhibits a reasonable correlation with velocity distribution. Constructed CFD models of dual drain tanks showed that increased side drain flow compared to the center drain flow decreases velocities and degrades vortex in the rearing tanks.

Tank geometry can also affect the hydrodynamics of the tanks and that is why we created models to account for this tank design characteristic. Our work showed that for small tanks (volume between 1-100 m³), the best performance in circular tanks can be achieved by the setup with a single inlet column that will produce a triangular shaped current behaviour. The best performance in octagonal tanks of the same size can be achieved by the setup with a single inlet column, where the direction of the individual inlet column will be in the middle of the shorter wall. For large tanks (volume between 100-1000 m³), the best performance in circular tanks can be achieved by the setup with two inlet column nozzles positioned at different angle. This arrangement of nozzles provides better velocity profiling and mixing pattern in addition to self-cleaning of tanks. The best performance in octagonal tanks of the same size can be achieved by the setup with two inlet column nozzles at different angle; either the inlet column is located at the start of the long wall, or the inlet column is placed between the long walls. Both designs, with their inlet placement and arrangement of nozzles develop better velocity profiling and mixing pattern across the large tanks with the addition of tank self-cleaning concept.

The same CFD modelling principles were applied on chosen prototypes of floating closed-containment tanks in the sea available in the



Centre Director of CtrlAQUA SFI Åsa Maria Espmark and Nofima researcher and WATERQUAL project leader Andre Meriac. Photo: Terje Aamodt, © Nofima

consortium. As a result, a more appropriate inlet configuration was suggested for FishGLOBE that is more efficient in flushing solid particles from the system.

Sensing of closed-containment environment has been in

focus throughout CtrIAQUA. Monitoring of water quality is an integrative part of closed-containment system management, however the sensor technology at the start of this project was not adequately developed. A severe problem industry was facing was formation of biofilms on the sensor surfaces after some use, which could change the sensor response and lead to incorrect readings. In CtrIAQUA three novel antifouling strategies



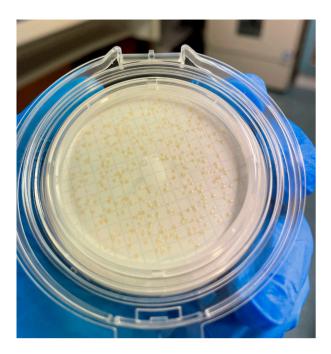
are explored, varying from nanocomposites based antifouling materials, fouling release materials to biomimetic microstructure materials. To expand lifetime and reliable sensor readings in aquaculture it is recommend using any of the developed antifouling materials for protection of sensor housings. This will also reduce time needed for maintenance of sensor systems. The most convenient and effective approach to combatting biofouling on sensor systems in aquaculture is nonbiocidal antifouling coatings that are nontoxic alternatives such as Polydimethylsiloxane/ zinc oxidegraphene oxide (PDMS/ZnO-GO) for antifouling coatings or Polytetrafluoroethylene (PTFE) coatings modified by O_2 plasma treatment which alters the microstructure of the film in favour of efficient antifouling properties. We have tested this approach by developing a membrane for CO₂ gas sensors with antifouling properties using Teflon coated with polydimethylsiloxane doped with zinc oxide-graphene oxide (PZGO). Membrane was tested in real system using authentic RAS water, indicating efficient prevention of biofilm coverage. however the response time of the sensor was increased by this modification. There is a need for further optimalizations, mainly connected to the thickness of the membrane that should be reduced to achieve faster response time of the sensor.

Real time monitoring of metals like zinc, copper and iron that could pose a threat to fish health if threshold values are exceeded is not currently available. Electroanalytical system was tested together with developed low-volume flowthrough cell in authentic RAS water to measure zinc, copper and iron continuously without addition of chemicals. Sensor has short response time, can be used at different salinities from approx. 0,1 ppt up to seawater (35 ppt). System is in progress to be optimized for typical concentration levels in recirculating aquaculture systems.

Dissolved organic matter (DOM) in RAS is poorly described and the changes in DOM composition caused by management of RAS, for example chose of feed or use of disinfectants in RAS loop (ozone or peracetic acid), are largely unknown. The use of RAS specific feed compared to standard commercial feed for Atlantic salmon lead to more efficient removal of several categories of DOM through water treatment process. The use of ozone in brackish water degraded DOM under transformation into relatively saturated molecules, with accumulation of nitrogen-containing compounds. Therefore, the levels of ammonia and nitrated should be carefully monitored during ozonation as observed increased levels were potentially caused by reactions of amines or amino groups with ozone. Organic matter in RAS water exposed to peracetic acid results in unsaturated molecules and CHOS, CHON aliphatic and aromatic compounds are formed. The potential impact on fish health and fish quality are still not fully understood.

Organic matter in RAS also affects risk of hydrogen sulphate H₂S formation potentially leading to mass mortalities, that have been observed in number of commercial RAS in the last four years of CtrIAQUA lifetime. Our results indicate avoiding the build-up of organic matter (sediments, biofilms) in RAS, to reduce the potential for H₂S production. Reduce organic matter loading in RAS, especially feed spill is crucial as one kg of feed spilled has a 6 x higher potential for H2S production than the faecal waste produced from 1 kg of feed consumed. In addition, nitrate delays and reduces H₂S response, but reduction of feed intake or increased water exchange can reduce nitrate concentration in RAS, potentially increasing the risk of H2S production.

Well-functioning biofilter is prerequisite for optimal operation of RAS. During postsmolt production in RAS industry is using increased salinity to prepare fish for the transfer to sea cages. However, salinity changes can negatively impact nitrifying bioreactors in RAS. This can lead to ammonia and/or nitrite accumulation, that can be





The objects of our UV tests: Tenacibaculum finnmarkense (upper image) and sea lice copepodit (lower image). Photos: Vasco Mota, Nofima.

toxic to the fish. We developed strategies for salinity change so that salinity changes can be made in a safe manner in RAS before fish are introduced: 1) Exposing the bioreactor to seawater in the startup phase (at least 2 weeks) and returning to freshwater before the introduction of the parr; 2) Startup in brackish water (12 ppt) followed by a freshwater phase before the fish are introduced; 3) Startup in freshwater with seeding with brackish water biofilm (12 ppt). Bioreactors that are to be operated at a constant high salinity (>12 ppt) can be started up directly at the operational targeted salinity. Nitrite oxidation can be challenging at high salinities. Thus, nitrite concentration should be closely monitored during and after salinity changes to ensure that it is within the toxic limits for the fish.

A key research line of the Department of technology and environment was semiclosed systems prototype development and testing. So far, all pilot S-CCS have been operating without treatment of intake water, however there was a need to evaluate the necessity and effect of such a treatment. Semi-closed systems and RAS operate on large intake water flows such as 100 m³/min. Available intake water treatment solutions for these large flows is scarce and require large investments. This lack of water treatment solutions can present a biosecurity risk through the transfer of natural occurring pathogens into these production systems. A low-pressure and monochromatic UVC lamp $(\lambda = 254 \text{ nm})$ and, medium pressure and polychromatic UVC lamp emitting at multiple wavelengths, (λ =220-300 nm) were tested to determine the required reduction equivalent UV doses to inactivate selected Atlantic salmon pathogens (two viruses: Infectious salmon

anaemia virus - ISAV and Infectious pancreatic necrosis virus - IPNV; three bacteria: Yersinia ruckeri - yersinosis agent, Moritella viscosa winter ulcer disease agent and Tenacibaculum finnmarkense - tenacibaculosis agent; one ectoparasite: Sea lice copepodites - Lepeophtheirus salmonis). Results indicate that use a UV dose of 25 mJ/ cm² is effective against all tested bacteria and viruses apart from IPNV. When IPNV is present in the water a UV dose needs to be higher and a mediumpressure UV is recommended. Also, UVC is not recommended to eliminate sea lice copepodites. A capillary polyethersulfone ultrafiltration membrane was also tested as a viable technology for removal of pathogens from the inlet water. We tested this technology against the infectious pancreatic necrosis virus - IPNV and the bacterium Aeromonas salmonicida. The results from this bench-scale study are encouraging for the application of ultrafiltration membrane technology in aquaculture water treatment to prevent virus and bacteria outbreaks.

Highlights of scientific results

In the centre description, CtrIAQUA defined 15 innovations that have been part of the annual plans every year. In the annual plan for 2023 we summarised the status of the innovations, and whether we consider them completed (Table 1).

Supportive Innovation	CtrlAQ	UA Innovations	Status / comment
Reliable, controllable, and efficient CCS technology	1)	Tank, bag, and raceway semi- closed containment systems	Completed: S-CCS`s tested by CtrIAQUA: Proof of concept regarding performance, health and welfare Water treatment (UV) for selected pathogens concluded.
	2)	Water quality sensor systems for high control level in CCS	Partly completed: 1) Implementing new membranes for sensor systems coating and toxicity studies of such membranes towards biofilm formation (Completed). 2) Implementation of sensor system for iron, coppe and zinc + development of a nitrate sensor system, will be concluded after CtrIAQUA termination (Partly completed).
	3)	Energy efficient RAS technology.	Completed: 1) Analytical procedures and protocols for analysing and characterizing dissolved organic matter. 2) CO ₂ degassing systems tested in RAS
	4)	Real-time waste feed and scale monitoring sensor for CCS; renamed to "Real-time biomass and scale monitoring sensor"	Completed: Due to changes in partner business model the innovation was renamed. VardAqua's AkvaVisior was proven successful for biomass estimation in S-CCS, and CreateView's camera technology was, in addition proven successful for scale and skir monitoring in S-CCS and flow through Implementation in RAS is pending
	5)	Real-time in situ water pathogen sensor for CCS	Partly completed: USN has developed a prototype of the FORDETECT (former POCNAD) technology: The technology remains to be verified.
Assessment tools and databases of requirements for Atlantic salmon in CCS	6)	Diagnostic assays for smolt and postsmolt performance and robustness in CCS	Completed: 1) Pharmaq Analytiq's SmoltVision, and 2) Nofima "health test": Diagnostic test that measures the activity of 44 genes that are important for the immune system.

Table 1. CtrIAQUA innovations and degree of completion

Supportive Innovation	CtrlAG	UA Innovations	Status / comment
	7)	Prediction models for optimal smolt transfer to CCS and post-smolt out	Completed: 1) Transfer models with different temperatures; 2) Optimal protocols regarding salinity, photoperiod and fish size at transfer; 3) Testing performance after low, medium and high water velocities.
	8)	A modelling database on environmental requirements of salmon for design and engineering of CCS	Partly completed: Due to IP issues we failed to collect data that were necessary for the database. Instead, a review of water quality limit values and some performance requirements from literature and CtrIAQUA experiments are compiled.
	9)	Robust Post-smolt Certificate	Completed: 1) Pharmaq Analytiq's SmoltVision, and 2) Nofima "health test": Diagnostic test that measures the activity of 44 genes that are important for the immune system
Tools to control pathogens, disease, and strengthen health of Atlantic salmon in CCS	10)	Diagnostic pathogen kit for CCS	Completed: New and/or improved qPCR methods for screening different pathogens have been developed. No new pathogens have been detected during CtrlAQUA time, as foreseen for this innovation. However, we have performed screening for different pathogens in RAS to investigate the origin of pathogens in S-CCS. Many pathogens seen in sea originate from RAS.
	11)	Pathogen Entrance and Health Protection System kit for CCS	Completed: 1) Screening for different pathogens in RAS to investigate the origin of pathogens in S-CCS; 2) CreateView machine learning of wounds and healing; 3) Low immune gene and thin skin right after sea transfer increase pathogen entrance; 3) Indications of evidence in favour of vaccine protection (Moritella viscosa).
	12)	Feed supplements for barrier protection in CCS	Completed: 1) Marine vs. plant- based diet: Dermis is thinner in the plant group. Marine diet effected the intestine by stimulation of blood flow, development and immunity. No difference in skin barrier function at sea transfer 2) No proven clinical protection against wounds or mortality cause by Moritella viscosa, but Zn in feed stimulated immune activity against other genes.
	13)	Guidelines for handling disease outbreaks, and biosecurity measures in CCS	Completed: Guidelines for use of UV, O_3 , PAA, O_3 + PAA, risk factors for nephrocalicinosis

Supportive Innovation	CtrIAQUA Innovations	Status / comment
	14) Vaccines against agents associated with CCS	Partly completed: During CtrlAQUA Pharmaq changed strategy from developing vaccines to vaccine protocols, since no new pathogens were observed. Indications of evidence in favour of vaccine protection (Moritella viscosa) have been detected.
	15) Guidelines for optimal vaccination and use of pharmaceuticals in CCS	Partly completed: we have not seen other or accumulated degree of pathogens in CCS, as hypothesised

Awards

Best student poster (H2Salar)

Hanna Ross Alipio, Julie Bergstedt Hansen, Nora Albaladejo-Riad, Carlo C. Lazado «Hydrogen sulphide (H2S) regulates the mucosal immune defences of Atlantic salmon *Salmo salar*». Aquaculture Europe 2022. Rimini, Italy. 27-30 September 2022.

3R Prize for Animal Research (Relevant CtrlAQUA project - EXPO, DISINFECT, TREAT and BARRIER)

Carlo C. Lazado and Elisabeth Ytteborg. 2021. Norecopa - Norway's 3R centre and National Consensus Platform for the Replacement, Reduction and Refinement of animal experiments.

Best student oral presentation (Cardio)

Nikko Alvin Cabillon, Gerrit Timmerhaus, Lill-Heidi Johansen, Carlo C. Lazado. «Health and welfare of Atlantic salmon (Salmo salar) subjected to different exercise intensities ».



Carlo C. Lazado and Elisabeth Ytteborg. Photo: Joe Urrutia, © Nofima

Mucosal Health in Aquaculture 2019. Oslo, Norway. 9-11 September 2019.

Best student thesis (Cardio)

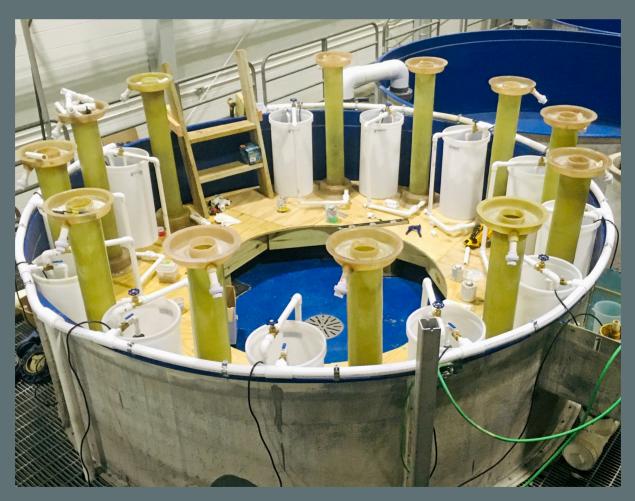
Nikko Alvin Cabillon « Plasticity of muscle growth and mucosal defenses in Atlantic salmon (Salmo salar) subjected to different exercise intensities». University of the Highlands and Islands, Scotland. 2018.

Best oral presentation (Preventive)

Tran-Minh, N. and Haug, B. A secure end-to-end IoT-based water sample collection system for early warning of attacks by sea lice larvae. 7th international conference on information and electronics engineering, Vietnam, February 25-27, 2018.

International cooperation

Researchers and user partners in CtrIAQUA have an extensive international network of contacts. In our Scientific Advisory Board, whose main task is to provide inputs to the annual plans and evaluate the scientific work, there are several international members, including from the European Aquaculture Society, Danish Technical University and the University of Aberdeen. CtrlAQUA researchers are invited as speakers at different international scientific meetings, and we are often invited to host sessions at meetings. During the European Aquaculture Society (EAS) conference 2022, arranged in Rimini, several CtrlAQUA related presentations were held. During October 2022, the seventh "Smolt production in the future" conference was arranged in Sunndalsøra, Norway. Nearly 300 participants from research, government, regulatory bodies, and industries met for the international conference. CtrlAQUA has a central role in this conference, both regarding presentations and arrangement / hosting. The Centre Director presented news from CtrlAQUA at the conference. Further, the Centre Director gave a presentation during "Seafood Talks, May 2022,



Experimental-scale biofilters under construction at The Freshwater Institute, to be used for a range of experiments involving disinfection compounds and their potential effects on biofilter performance, which is vital for successful RAS operation and maintaining fish health. Photo: Christopher Good, © Freshwater Institute

CtrlAQUA

where CtrIAQUA was part of the theme "fish welfare and environmental challenges in closed-containment systems".

There are two international R&D partners in CtrlAQUA, Gothenburg University (UGOT) and The Conservation Fund Freshwater Institute (FI), USA. Gothenburg University is represented in CtrlAQUA by Prof. Kristina Sundell and her research team. UGOT has in 2022 contributed to important knowledge regarding skin and gut as barrier organs. They have also continued their work in an associated project on effects of microplastics in RAS. The Conservation Fund Freshwater Institute (FI) is represented by Dr. Chris Good and Dr. Brian Vinci with colleagues. They are heavily involved in the work with sexual maturation, disinfection protocols and hydrodynamic modeling. Both Chris Good and John Davidson contributed with presentations at the "Smolt production in the future" conference.

CtrlAQUA opened for associated projects in 2015. Associated projects need external funding and can in addition to CtrlAQUA partners involve partners that are not regular CtrlAQUA partners. In 2022 we have had five associated projects that involves international partners:

1) "Microplastics in the environment" led by UGOT and funded by Swedish Research Council: an investigation into how microplastics affect fish and potential risks for the aquaculture industry.

2) "Prevalence and consequences of hydrogen sulphide in land-based Atlantic salmon production" (H2Salar), led by Nofima, funded by RCN. The primary objective is to create knowledge and advance the understanding of the risks and impacts of exogenous hydrogen sulphide (H2S) to the physiology of Atlantic salmon in recirculating aquaculture.

3) "Water disinfection strategies to improve Atlantic salmon parr production in freshwater recirculating aquaculture systems" (RASHealth), led by Nofima, funded by RCN. The primary objective is to optimize existing disinfection protocols and develop new water disinfection strategies to control pathogens in Atlantic salmon freshwater RAS.

4) "The balancing act: Biologically driven rapidresponse automation of production conditions in recirculating aquaculture systems (RAS)" (RAS 4.0), led by Nofima, funded by RCN, where the main objective is to improve fish wellbeing and production efficiency and reduce operational risks by developing integrated control systems for water quality, feeding and energy optimization. The control systems are based on novel sensors, data integration and smart algorithms that combine biological, environmental, and operational factors.

5) "Kunnskapskartlegging produksjon av stor laksesmolt", led by Nofima, funded by FHF, where published and experience-based knowledge about production protocols for postsmolt that are used in Norwegian salmon farming and the other relevant salmon farming countries, including the Faroe Islands are collected and disseminated.

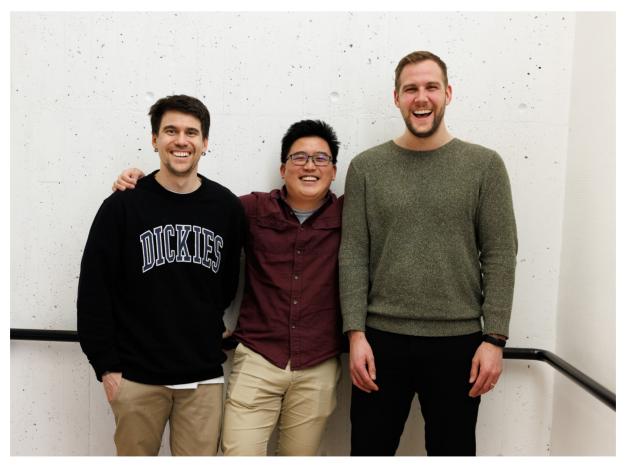
Researchers in CtrlAQUA are often involved in new project proposals where international partners are included. One example is a series of EU projects where some of the CtrlAQUA-partners have helped establishing first "AQUAEXCEL", then secondly "AquaExcel2020" and now the ongoing "AQUAEXCEL3.0", where, amongst others, Nofima Centre for Recirculation in Aquaculture (NCRA) in Sunndalsøra is included as one of the Transnational Access Points (TNA), TNA means that researchers across Europe can do experiments in NCRA funded by AQUAEXCEL3.0, as was also the case in AQUAEXCEL and AquaExcel2020. Also, both NORCE and Nofima coordinates one EU project each under the call "DT-BG-04-2018-2019: Sustainable European aquaculture 4.0: nutrition and breeding; iFishienci and FutureEUAqua, respectively.

Training of researchers

At NTNU, four PhD candidates have been involved in CtrlAQUA. Among the four candidates, one had an industry PhD. All of them were females where to were two were recruited internationally from China and Spain while two were recruited from Norway. Except for the associated industry PhD, all the three positions were announced in English to reach out as broad and internationally as possible. However, national recruitment was also done by inviting potential talented candidates to apply. This included

informing relevant professors at Norwegian Universities about the announced positions so potential talented candidates could be reached outside NTNU. Relatively many of the master students were recruited from NTNU, and all of them had project building in existing research activities in the centre. In general, the master projects included close contact with at least one other partners in the centre. Master students also joined the annual centre meetings where they presented results from the projects as well as having

the possibility to meet the industry partners in the centre. The centre have especially added valuable contributions to the educational programs by offering relevant master projects in collaboration/ relevance to industry. This helped to fulfil goals in Meld. St. 5 (2022-2023) Longterm plan for research and higher education 2023-2032 and helped to align with Tilldelingsbrev NTNU and NTNU strategy stating that the research and study programs shall support sustainable innovation with basis in centres.



CtrIAQUA students (from left) Patrik Tang, I-Hao Chen and Marius Takvam. Patrik and Marius are now employed by CtrIAQUA R&D partner NORCE. Photo: Terje Aamodt, © Nofima

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At UiB, a total of five PhD students and one dr. philos have been associated with the institute. Four of the PhD students have been recruited internationally as with the dr. philos. Of these, three were male and three were female. All of the positions were announced nationally and internationally, and according to regulations, the candidate with the best qualifications was hired. UiB has had a large number of master students overall associated with the centre, representing an investment in research-based teaching. Master students have represented an important resource for the centre, and the students have been associated with practically all projects within it. The integration of master students has represented a work force for carrying out the projects, and the CtrIAQUA has provided opportunities for the students to address important topics. We see the large number of students as a success criterion for the centre.

The following selection of candidates are examples from interviews done for the annual reports where we asked about their work and motivations.

<u>Xiaoxue Zhang</u> did her PhD student at NTNU Nanolab from 2016.

Before her arrival at CtrlAQUA, closed-containment aquaculture was completely new to Zhang but, once she had started, she soon developed an enthusiasm for the work.

"At first, this was just a job that suited my competence. But, once I was involved, I saw how passionate the others at the centre were about their work and it was contagious. I am now very enthusiastic about developing this material to improve the sensors," she says. Later, Zhang also made a film describing her research; https://playei

Photo: Per Henning, © NTNU

made a film describing her research: https://player.vimeo.com/video/334421466?h=Ofe4194fd2

Ingrid Gamlem did her MSc degree in CtrIAQUA at the University of Bergen in 2017. "To make closed systems a reliable and economically viable technology, my contribution will be to clarify the production potential in a semi-closed system. These innovative systems have the potential to solve some of aquaculture's major challenges, whilst also being vital to the industry's ambitious future. I absolutely want to continue to work in this field if the opportunity comes my way," says Gamlem.

<u>Sharada Navada</u> is a process engineer employed at Pure Salmon and did her PhD at NTNU and Nofima.



Photo: Eivind Senneset Bergenhus/UiB

When starting her PhD, she imagined that she would continue working for the recirculating aquaculture system supplier, which she is:

"I hope that during my four years as a PhD student, I will be able to build a good knowledge base, forge social connections in the scientific community and develop myself, so that I can contribute a lot more when I return to industry." Navada is very optimistic about closed-containment aquaculture:

"I believe that once we have improved the biofilters, we should also be able to reduce freshwater consumption quite substantially. That could have an economic impact by reducing the energy required for pumping and temperature regulation." Navada also made a film to describe her work: <u>https://player.vimeo.com/</u> <u>video/252868239?h=f3c57e333f</u>

Patrik Tang is a PhD student who is linking several projects in CtrIAQUA.

Photo: © Pure Salmon Technology

In Tang's opinion, the technology for closed-containment aquaculture is still not complete and has a somewhat long road ahead. However, Tang is a firm and positive believer that in the near future, as these systems develop and are made more efficient at lower costs, they will become a viable solution to many issues associated with aquaculture.

"For these reasons, I am deeply privileged and grateful to be a part of such an important and innovative movement", he says.

Raised in Bermuda off the coast of Florida, he moved to Finland where he studied Physiology and Neurosciences at the University of Helsinki. After his studies, he worked with fish and seal populations of the Baltic Sea, within the field of nutrition and physiology. It was quite a leap when moving to Bergen to work with closed and semi-closed containment systems for salmon:

"To be honest, before coming to Bergen, I didn't have much of an idea about closed-containment aquaculture. However, so far it has been an amazing experience and an overwhelming field to be associated with and learn about! My supervisors have been great, additionally



Photo: Terje Aamodt, © Nofima

the people I come into contact with working at farm sites or generally in this field are inspiring, not to mention, the people I sample with on site, who teach me so much valuable knowledge. Such has taught me that key is good communication among different parties involved, including both the science community as well as the people working at the farms, aspects that I find essential in achieving the common goal", says Tang.

Lene Sveen, who is now a fish health researcher at Nofima, defended her PhD in 2018 at the University of Bergen.

She found that when a fish becomes acutely stressed, it loses its protective mucus coating. And it takes more than a day for it to return. This is bad news for the fish, as the mucous coating protects the fish against diseases.

"My message is that we must focus on preventative fish health when designing closed-containment

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systems. I think it will be very interesting to see how the industry addresses this challenge."

"Closed-containment aquaculture systems are evolving rapidly, and I can't wait to see what the fish farming industry's equivalent to the iPad will be," says Sveen.

John Davidson is an experienced researcher who stands out from the classic research fellow. He has had a long research career at the Conservation Fund Freshwater Institute in the US. In June 2021, he defended his Dr. philos. degree through the University of Bergen.



Photo: Jon-Are Berg-Jacobsen, © Nofima

Now that you look back, what is your best advice for the aquaculture industry?

"Successful production of Atlantic salmon and other species in RAS requires multifaceted expertise amongst a team that is well versed on a range of topics including: fish biology, water chemistry, systems engineering, operations, food science, and waste management, among others. This wide scope of understanding has kept research and development of land-based RAS constantly interesting to me. There is always something new to learn and discover. As such, additional research is constantly required to support this rapidly growing aquaculture

What is your take home message for future research needs?

sector."

"Over my 20+ year career, I have seen RAS research needs shift slightly from optimizing engineering design and related water quality to other niche topics. For example, developing solutions for off-flavor and evaluating holistic effects of newly developed diets for RAS are now at the top of my list. At the Freshwater Institute, our team is also working on precision aquaculture initiatives such



Photo: © Pure Salmon Technology

as fish biomass estimation through camera vision and machine learning and waste-to-value research that is focused on turning fish waste into valuable products such as biogas and compost. It's an exciting time to be involved with the RAS industry!"

Employment of CtrlAQUA PhD candidates

Employmen	Employment of PhD candidates (number)													
By centre	By other	By public	Ву	Ву	Outside	Other	Total							
company	companies	organisations	university	research	Norway									
				institute										
2	1		1	2	4		10							

Communication and dissemination

The centre and its partners have been very visible in Norway and internationally, with a total of 382 articles in media, and hypothetical reaching an audience of 8 million people. In the aquaculture and scientific community, the interest from the media has been quite stable throughout the centre lifetime in Norway. Internationally, the interest from the media has been increasing throughout the period, growing along with the rising activity in the industry. Based on media analysis from Infomedia, about 2/3 of the media coverage has been in Norwegian, and 1/4 in English. Partners in the centre have been popular in debates,

podcasts, and seminars covering closed-containment aquaculture particularly in Norway and USA.

In the communication plan for CtrIAQUA, the general public was defined as the tertiary target group after the aquaculture industry and academia. The effort of targeting this group has therefore not exceeded that of the other groups. At start up in 2015, CtrIAQUA had a large stand at the "Forskningsdagene" in Oslo city centre, interacting with the public about the possible shift in fish farming methods towards closed pens. CtrlAQUA.no shows a quite high news production of 63



articles through the lifetime of the centre, some of which are blogposts, student films, and news posted at Forskning. no for the general public. The communication department has also conducted several interviews with CtrlAQUA people within industry, research and students, not only talking about the output, but sharing their thoughts of aquaculture that can be interesting for a wider audience. Good, professional images of the activity have also made this news more relevant to the press.

The Centre Director and scientists have always made themselves available when the media have asked for interviews, generating news in public media such as Aftenposten Innsikt. They have also been well represented during the biannual international conference "Future smolt production" held at Sunndalsøra and organised in collaboration with Nofima and Sunndal Næringsselskap.

At the conference "Smolt production in the future", participants from the entire value chain were invited to visit the RAS facilities of Nofima, and to hear about research in CtrlAQUA. Photo: Frode Nerland, © Nofima.



Looking back and going forward

Effects of centre for the host institution and research partners

CtrlAQUA was in line with the host institution, Nofima's, research strategy before the centre start and is still an important contribution to the strategy regarding securing health, welfare and performance in existing and new production technologies, and working towards sustainable and environmentally friendly future aquaculture, amongst others. The biological and technological development of the closed-containment solutions, RAS and S-CCS have made Nofima one of the most important R&D contributors to the industrial development of these solutions. One



In 2022, Nofi ma opened the new RAS facilities with one tank / one RAS systems in Sunndalsøra. We managed to perform the last CtrlAQUA trials in the new systems, and together with the rest of the Nofima RAS infrastructure, these systems will enable us to continue to provide relevant RAS research for the industry. Photo: Terje Aamodt, © Nofima

CtrlAQUA Simil Report 2023

important R&D contribution is to secure animal welfare along with the technological development, and to see that the technologies fulfil the biological needs of the fish. All R&D partners share the strategy on welfare and technology development and the collaborations continue in new projects and applications.

The R&D partners responded that being part of CtrlAQUA has significantly broadened their research network and resulted in many new collaborative constellations. The research partners within CtrlAQUA have many complementing competences. This has led to opportunities in designing truly interdisciplinary experiments and designs that have been able to investigate research questions and applied challenges from a broader and more holistic viewpoint, resulting in more solid and societally applicable results. They believe that the transdisciplinary format of the centre of very close contact and collaboration with the industry is the main added value of CtrIAQUA. It has made it possible for researchers to build sound scientifically based hypothesis based on actual bottle necks, challenges and questions within the industry, and in a very rapid and efficient way to implement the results from the research directly.

Regarding whether the centre's activities have contributed to renewal, increased quality and reduced fragmentation in the Norwegian research



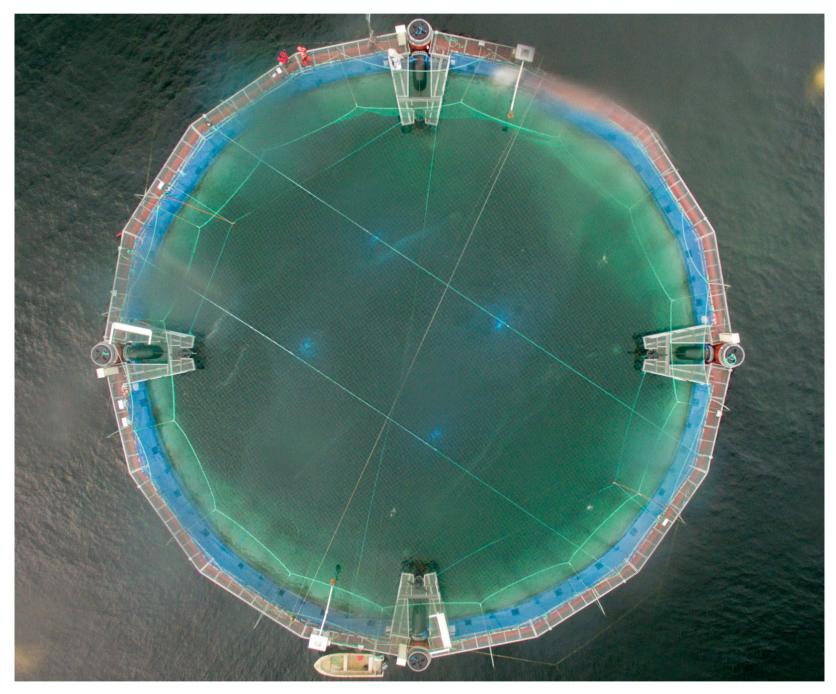
NTNU PhD students Xiaoxue Zhang, Sharada Navada, Ingrid N. Haugen and Patricia A. Alarcon with Professor Øyvind Mikkelsen in the middle. Photo: Per Henning/NTNU

system, the R&D partners responded that they believe that the centre has contributed to less fragmentation in the Norwegian research system. Further, the centre has added valuable contributions to the educational programs by offering relevant master and doctoral projects in collaboration with the industry. This helps to fulfil goals in Meld. St. 5 (2022-2023) "Longterm plan for research and higher education 2023-2032" and helped to align with the university's strategies stating that their research and study programs shall support sustainable innovation with basis in centres. The centre has served as an excellent platform for networking both for master students, PhDs and

researchers. CtrlAQUA has contributed significant with the education of students to meet the need for, especially RAS competence, that has been requested for many years both nationally and internationally. The centre's 16 PhD and almost 60 MSc students are either already employed or on the way to be employed in R&D or industry companies, nationally and internationally.

Effects of centre for the company partners, public partners and society at large

The user partners have responded to questions regarding how the centre has affected the industry partners, public partners and society at large. How have the centre's activities contributed to innovation and value creation at the participating partners? The partners reported back that CtrIAQUA has helped the industry to safeguard the development and implementation of RAS technology and S-CCS for



Current Certus semi-closed containment system (8400 m3) located at Cermaqs site at Horsvågen, North Norway. Photo: Tor Evensen/Nofima

CtrIAQUA Sinal Report 2023

smolt and post-smolts that is a part of the industry production platforms today. More specifically, they mention contribution to technology and RAS process improvements, fish tank optimization and production improvements (e.g., smoltification protocols and strategies). Optimizing water quality in RAS for smolt and post-smolt in regard to welfare and performances, microbiota management in RAS as well as performances after transfer in net pens were also mentioned as specific contributions. They concluded that CtrIAQUA has supported collaboration between industry partners and improved knowledge exchange.

2. What type of value has already been realised?

The S-CCS partners have used the monitoring of performance, health and welfare in their further production and commercialisation of the different systems. They also use the computational fluid dynamic (CFD) modelling when designing new systems.

The farmers reported of improved smolt production by survival, better fish health and welfare, and increasing growth. One partner specified that they have increased the smolt sizes over the last 8 years making a shorter production time in net pens possible. More than 50% of their smolts are now produced in RAS or S-CCS systems. They have also used the centre results to reduce risks in closed-containment, with e.g., H₂S. They have also optimized water intake disinfection, applied ozone in RAS, and implemented recommendations on biofilter management during salinity changes.

3. What is the outlook for future value creation potential?

CtrlAQUA has established a solid platform for future research and development for further improvement and finetuning of the smolt production. The industry will continue to improve their operations using RAS and S-CCS technologies. They foresee that the knowledge created in the centre will contribute significantly to their efforts to produce smolt and post-smolts in an even more cost effective and sustainable way. They believe that this will have a positive impact in the whole value chain. There is potential to increase value creation in the future within both post-smolt production and land-based up to slaughter. This requires good solutions and design for the industry with a strong focus on fish welfare and health, as well as optimizing resources such as water and energy.

4. How has the centre helped to enhance the competitiveness of the participating partners?

The industry partners strongly believe that CtrIAQUA has lifted Norway up amongst the most comprehensive and attractive research partners for RAS and S-CCS for Atlantic salmon. The many students produced throughout the SFI program, have and will continue to contribute in the future to lift and develop the supply industry, production companies as well as R&D entities. The centre has contributed to increasing the general knowledge within the production of salmon in closed and semi-closed facilities and in that way added valuable knowledge to aquaculture production in the country. Lastly, the partners agree that centre meetings, networking and discussions have been very important for the competitiveness of the participating partners.

The industry partners ranked what was considered the most important effects of participating in the centre. The most important reason was "Improved network to other partners", followed by "Strengthened knowledge base for the partners". They also voted for "Improved access to competent personnel and research institutions", "Development of new or improved products, processes or services" and "Influence on R&D and innovation strategy of the partners". Other key arguments are "Improved sustainability of products, processes or services". None of the respondents voted for "Recruitment of qualified personnel" or "Positive environmental effects". These answers are very much in line with the answers given during the mid-term evaluation.

Success stories

CtrlAQUA partners have expressed the unique social environment in the centre and the importance of the networking. We have experienced that at the start of the centre, both RAS and S-CCS were in the early days of development, and the user partners were very open with their plans, results, and experiences. Many have expressed the positive effect of discussing common issues with equals in a long-lasting project where project results have resulted in significant technological development. CtrlAQUA has produced an extensive number of results, and among many others that have received much attention in the consortium and been implemented are:

 The development of smolt production protocols under different environmental conditions that have resulted in that many farmers dropping the winter signal. As a result, some report of improved growth and reduced mortality during the RAS phase.

- Better water quality due to improved biofilter performance in RAS.
- Establishment of RAS specific velocities for postsmolt led to stronger focus on flow velocities in tanks.



Sampling. Photo: Terje Aamodt, © Nofima

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- Hydrodynamic modelling of inlet pipes to improve water distribution. The project increased focus on inlet pipe tuning and flow control for reduced turbulences, more even velocities and improved self-cleaning.
- RAS specific limit values for water CO₂ levels in tanks led to increased focus on tank exchange rate, need for CO₂ stripping, and sensor control.
 Implementation of adapted protocols and intensified CO₂ monitoring.
- Increased focus on preventive methods to avoid H₂S caused mass mortalities. Implementation of adapted protocols for H₂S monitoring.
- Diagnostics and evaluation of nephrocalcinosis using histology, X-ray and manual macro scoring.
- Understanding the ways of pathogen transfer into S-CCS systems and that many pathogens are transferred via smolt from the hatcheries.

The most important CtrIAQUA results have been summarised in 42 factsheets, where also highlights for industrial needs and recommendations are given. Each factsheet suggests level of readiness for implementation.

One feedback quote from the industry partners:

"RAS technology have now become a more "of the shelf technology" in the SFI life span. RAS is now proven to work for smolt production, even though the cost issues for investment needed in systems and an energy cost remains to be improved. In the beginning of the SFI period, RAS technology had a lot more questions and uncertainties. Most of these issues have been addressed in the centre or in associated projects, throughout these 8 years, leaving us in a much better position today when it comes to understanding and the use of the technology. On the other hand, the S-CCS technology for post-smolt have gone through much of the same journey, leaving us with a better insight now of what remains to be solved. The different speed of development for RAS and S-CCS reflects the regulatory regime that have been applied in Norway in the centre lifetime and is not only limited by the SFI's efforts to bring S-CCS up to the same insights as RAS".

The networking between partners within and between categories have resulted in R&D partners visiting industry partners and other R&D partners during the centre time for project work, but also for developing new ideas that has resulted in new projects. Many of these projects have been taken up as associated projects to CtrIAQUA, meaning external funding outside CtrIAQUA, and also involving partners outside the consortium. Examples of such projects

include the sea phases of two life-cycle projects to develop smolt protocols (FHF funded), development of knowledgebase for large smolt production protocols (FHF funded), understanding effects of H2S and development of indicators for H₂S (RCN funded), and establishment of control loops using sensor technologies in RAS (RCN funded). Common for all associated projects are to give opportunity for adds-on to CtrlAQUA to develop even more knowledge.

Patents, commercialisation and new business activity

Below is a short list of patents achieved by some partners. Table 1 summarises

University of South-East Norway:

Data from University of South-Eastern Norway (USN) used in the CtrIAQUA project PREVENTIVE (FORDETECT), has been used for the patent application PCT/ EP2022/058869 (patent nr WO/2022/218739), submitted 14 April 2021.

We would like to conclude this chapter with a couple of quotes from two industry partners that very much summarises what also the host institution and leader group believe represents the consortium view about how it has been to be a partner of CtrlAQUA during the 8-year centre time:

"We believe that being part

CtrIAQUA innovations, that are methods, procedures and / or technologies that are mostly

FishGLOBE:

- NO 20150884 / NO 339207

 Closed tank for fish farming and methods for transporting fish in and from the tank.
- NO 20161238 / NO 340479
 Methods for transporting fish in and from a closed tank.
- Results based on CtrIAQUA proof of concept done in FishGLOBE.

of an SFI entre for RAS and S-SCCS in this period of time (2015-2023) have been very important as a backbone for our insights in and use of these technologies. The regularity of the meeting places, the students, the internal presentations and the peer-review publications have been important instruments to secure this". either commersialised or just about to be.

Pharmaq Analytiq:

SmoltVision, the realtime PCR analysing tool to ensure that fish is smoltified and ready to be transferred to sea water, has been developed in cooperation with CtrIAQUA

"Interacting and being challenged by industrial salmon producers in a different battle filed than business related, building knowledge together and confidence becoming a valuable partner for academic and R&D centres for projects and research".



Illustration and real-life photo of FishGLOBE producing post-smolts in Lysefjorden. Photo: © FishGLOBE.

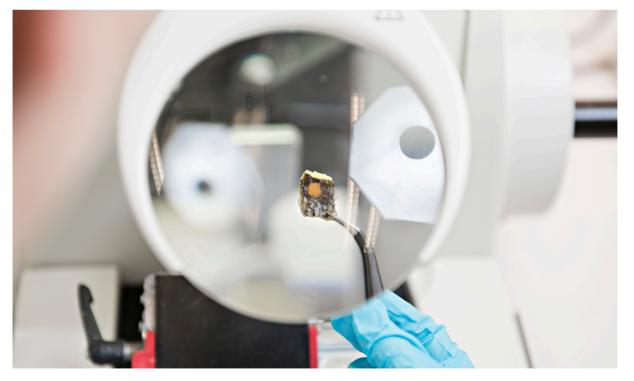
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Future prospects

The host institution, Nofima recognises the expectation of having a special responsibility to maintain the expertise acquired and safeguard the investments made during the centre's period of operation. It was also one of the recommendations from the Mid-term evaluation from 2019 to plan for after the centre lifetime. As previously mentioned in this report, the CtrIAQUA partners have repeatedly expressed their positive experience in working together in long-term projects towards common goals and being able to discuss and plan for solutions to common interests. They agree that it is possible, even for partners who otherwise are competitors, to cooperate in important

issues with common interests. However, it is important to realise and accept that cooperation is not always possible for all competing issues, and to address these are important for fruitful cooperation.

In CtrIAQUA we started the discussions about "post-CtrlAQUA" already in 2021. During two meetings the consortium met to discuss future collaboration and plans for further projects and possible fundings. During the two meetings, we came to the realisation that the consortium did not manage to agree in common interests for future projects that involve all partners. Hence, we will continue with the consortium members in different projects, according to their interest. This has resulted in two FHFfunded projects where user partners are involved. We have also initiated plans for larger projects, where one involving many of the consortium partners has been submitted and another initiative is in the process of being developed. Also, all 21 partners have signed a supplementary consortium agreement that secures the partners rights to unpublished CtrIAQUA results, and the rights to physical project materials that are stored at partner institutions. We will thus keep the intranet site for at least five more vears. This site also enables further communication and dissemination involving the partners.



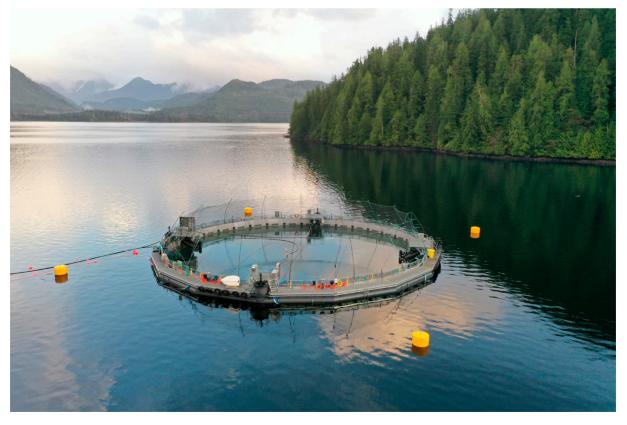
Skin health and wounds on farmed salmon has been one of the many research areas in CtrlAQUA. Photo: Jon-Are Berg-Jacobsen, © Nofima.

Conclusions

For Nofima, CtrlAQUA was the first SFI as host institution. It has been an interesting journey for all involved, and the general conclusion is that for Nofima, CtrlAQUA has been a successful project and learning process. One success criterion was that the centre topic of closed aquaculture systems was very hot when the centre started in 2015, and this created a lot of national and international interest. The topic has been of high interest throughout the centre and both researchers and Centre Director have presented CtrIAQUA and results at several national and Internationale meetings and in the media. Closed aquaculture systems, optimising the technologies and securing fish

health and welfare in them, were very much in line with the Nofima strategy from the start of the centre start, and they still are. The strength of Nofima is that we have the necessary support functions that are required for running a big centre like CtrIAQUA. In retrospect, it is clear that managing such a big centre demands devoted controllers, communication staff and IT personnel. CtrIAQUA has also had access to one devoted Centre Coordinator that has been in charge of all reporting and meeting organisations and minutes. This has almost been a full-time job. The two main R&D partners, Nofima and Norce have also been members of the Board.

The SFI model has been successful for the involved CtrlAQUA partners. The scientific leader-group, consisting of Centre Director, Centre Coordinator and four department leaders (Department of Technology and Environment, Production and Welfare, Preventive Fish Health, Training and Recruitment) and one member from the second largest R&D partner, has gathered virtually every third week and physically twice a year, in addition to the annual meetings. This frequent contact has enabled continuous following ups and decision makings. The Board has met two times every year. The first meeting has been in association with the annual



Angle aerial of Certus S-CCS from Sam Chen, Canada. Photo: © Cermaq



Salmon smolt recently transferred to sea water. Photo: Terje Aamodt, © Nofima

meeting in May, while the second meeting has been in November, where major items have been approving the annual plan and going through the project status. The CtrlAQUA Board has throughout the centre time been a successful help to the project and a solid base for advice, guidelines and constructive decisions in important cases. Also, the scientific advisory board has been very devoted and provided constructive feedback to the centre progress. The annual plans have been created in a step-by-step process. The initial discussions have been during the annual meetings, but the content of the annual plans also came

from the centre description, the defined innovations, and the yearly one-to-one discussions between Centre Director and each of the partners. We have continually explained that active partner involvement has resulted from engaged partners that have participated in annual meetings and 14 thematic meetings. We believe that much of the positive involvements are results of that the management group has followed the request from the industry partners to present results in easily accessible ways, such as presentations at annual meetings, in thematic meetings and at the partner facilities. Annual and thematic meetings have also invited the students. All PhD students

have presented their work in one or more meetings, and many master students have also presented their work. The students have been able to present their work for the industry partners, and they are attractive candidates for many of the partners businesses. To conclude, CtrlAQUA has been a successful centre for all involves partners. We have learned much, and we are convinced that we have contributed with important knowledge regarding system development, and health, welfare and performance optimisation in closed containment systems, to the benefit for the aquaculture society.

Financing through the life of the centre

Summary sheet for the main categories of partners (NOK million)

Contributor	Cash	In-kind	Total
Host (Nofima)		23	23
Research partners		13	13
Companies	37	109	146
Public partners		13	13
RCN	96		96
Sum	133	158	291

Distribution of resources (NOK million)

Type of activity	NOK million
Research projects	261
Administration	30
Total	291

Results - Key figures

	2015	2016	2017	2018	2019	2020	2021	2022	2023	Total
Scientific publications (peer reviewed)	1	8	6	11	12	12	11	18	4	83
Dissemination measures for users	17	33	36	59	31	19	9	31	6	241
Dissemination measures for the general public - Popular science publ.	1	3	3	0	5	3	6	5	1	27
PhD/post-docs/ dr. philos degrees completed			2	1	2	0	4	5	7	21
Master degrees		2	6	1	9	9	14	10	9	60
Number of new/ improved methods/ models/proto-types finalised		1	1	2	0	0	3	9	2	18
Number of new/ improved methods/ products/processes/ services finalised				2	6	0	1	3	1	13
Patents registered			1		1		1	1		4
New business activity								1		1



Statement of accounts for the complete period of centre financing

Funding (1000 NOK)

Total funding	22194	11987	12660	13456	0069	24287	10610	8384	14984	19721	16449	6499	84653	2982	6629	1201	1888	1434	544	4540	2501	1212	192	185	848	9342	5018	291300
Partner 24 - Pure Salmon Kaldnes	73	46	46	35	65	33	70			178		350	110															1006
Partner 23 -Atlantium	32	31	28	17	49	68	28	229					12	20												2		516
Partner 22 - F CreateView -	86	42	200	19	06	126	65			7	100	75	399	3												33		1245
Partner 21 - Pa Univ Sørøst Cr Norge									381				132	10														523
Partner Pa 20 - FiiZK Ur	25	32	34	15	24	64	37	182		120	5		357	10	4													606
Partner 19 - FishGLOBE 2	21	40	37	34	24	116	84	006		206	145	26	467	10	19	2					64					3.62		 2557
Partner Pa 18 - Fi Aquafarm	125	49	120	50	20	26	95	613			113	46	164	10	19	-					64					301		1823
Partner 17 - Nekton	43	394	110	50	1335	213	87		912	588	150		1888	10	16	80		644			64							 6512
Parner 16 - Vard Aqua	41	164	83	50		36	122				50	90	15	988	50	22	258				94		06			115		 2268
Partner 15 - Oslofjord	570	196	105	191		40	159		1141		1127	146		25	195	28					91	357						4371
Partner 14 - Pharmaq	136	290	472	191	107	335	298	4	298	644	860			35	257	17					06	206						4240
Partner 13 - Pharmaq Analytic	176	297	480	191	121	244	298	4	296	255	111			35	124	17					06	184						3589
Partner 12 - Kruger Kaldnes	26	227	127	157	70	172	209	104		255	250	425		35	169	44	234			916	94					771		4356
Partner 11 - Bremnes	635	462	304	175	263	499	357	25	538	1536	136	17	118	75	93	30				289	107				141	233		6033
Partner 10 - Cermaq	585	467	307	175	250	518	369	612	2231	1032	418	323	853	75	375	28	68				107					141		8934
Partner 9 - Lerøy Seafood	745	470	309	175	288	586	491	332	640	1171	262	200	6814	75	93	27					107				210		2848	16178
Partner 8 - Grieg Seafood	677	462	301	175	262	926	381	25	522	1146	302	50		75	93	32	222			538	107					229		6525
Partner f 7 -Mowi ASA	735	467	305	175	267	555	440	25	757	1426	373	400	67789	75	314	26			352		107					11		74599
Partner 6 - University of Gothenburg		28	476			26			56	75																		661
Partner 5 - NTNU				6054																								6054
Partner 4 - Freshwater Institute			10			1667	292					245								120	193			10		347		2884
Partner 3 - Universitetet i Bergen	978	30	42			2028				141	5839		69								17			12	17		83	9256
Partner 2 - NORCE	627	1070	73			944	34	25	52	161	50		907		1243	78			92	311	384			÷		16	1346	7424
Host - Nofima	6437	133	2161	87	965	965	1524	1124	1930	2150	77	796	589	496	725	44	206	290		566	21	165	22	22		1181	161	22837
RCN	9350	6590	6530	5440	2700	14100	5170	4180	5230	8630	5080	3310	3970	920	2840	790	006	500	100	1800	700	300	80	130	480	5600	580	00096
ltem	Project 1 - Management	Project 2 - Robust	Project 3 - Barrier	Project 4 - Sensor	Project 5 - Waterqual	Project 6 - Optimizie	Prosjekt 7 - Hydro	Project 8 - Intake	Project 9 - Preventive	Project 10 - Benchmark	Project 11 - Microparasites	Project 12 - Disinfect	Project 13 - Rigid S-CCS	Project 14 - Biomass	Project 15 - Co2Ras	Project 16 - Database	Project 17 - Expo	Project 18 Flexibag	Project 19 - Neptun	Project 20 - Particle	Project 21 - Photo_FW	Project 22 - Pocnad	Project 23 - Removal	Project 24 - Risk	Project 25 - Transfer	Project 26 - Treat	Project 27 - Preline	Sum

Total cost	22194	11987	12660	13456	0069	24287	10610	8384	14984	19721	16449	6499	84653	2982	6629	1201	1888	1434	544	4540	2501	1212	192	185	848	9342	5018	291300
Partner 24 - Pure Salmon Kaldnes					286																							286
Partner 23 -Atlantium								255																		2		257
Partner 22 - CreateView			195							7			308													33		543
Partner 21 - P Univ Sørøst C Norge									381				142															523
Partner U 20 - FiiZK								111		120			70															301
Partner 19 - FishGLOBE 2								575		267	134	26	475													362		1839
Partner P ₆ 18 - F Aquafarm	88							479			62		174													301		1104
Partner 17 - Nekton		331			1317	146			1206	462			1530					800										5792
Parner 16 - Vard Aqua														581			150						64			115		910
Partner 15 - Oslofjord	521								086		1703											289						3493
Partner 14 - Pharmaq			337			88			35	517	491											139						1607
Partner 13 - Pharmaq Analytic	48	108			2				33	189	457											117						957
Partner 12 - Kruger Kaldnes	9				18		28	66		42		24					219			931						171		2168
Partner 11 - Bremnes									277	635		17	118							59					51	233		1390
Partner 10 - Cermaq								739	1755	550	353	23	731													141		4292
Partner 9 - Lerøy Seafood	413							16	89	87	36		6516												189		4189	11535
r Partner ri 8 - Grieg Seafood	455		6		4	405			122	357	1 2									307						9 229		1881
- Partner of 7-Mowi rg ASA	435	9 128		14	13	4 35	80	28	335 374	4 807	73		67687						263									8 69955
Partner 6 - University of Gothenburg		129	2586			194			33	294																		3538
Partner 5 - NTNU				13123																								13123
Partner 4 - i Freshwater Institute			20			5761	1790					2768								32	1312			62		1158		12903
Partner 3 - Universitetet i Bergen	515	16	207			4171				2	12564		496								18			33	17		10	18052
Partner 2 - NORCE	5740	10817	096		156	8313	792	976	1662	3337	231		4613		2725	1083			281	593	972				591	106	774	44722
Host - Nofima	13973	458	8346	319	5101	5174	7890	5106	7735	12045	343	3641	1793	2401	3904	118	1519	634	0	2618	199	667	128	60		5882	45	90129
Item	Project 1 - Management	Project 2 - Robust	Project 3 - Barrier	Project 4 - Sensor	Project 5 - Waterqual	Project 6 - Optimizie	Prosjekt 7 - Hydro	Project 8 - Intake	Project 9 - Preventive	Project 10 - Benchmark I	Project 11 - Microparasites	Project 12 - Disinfect	Project 13 - Rigid S-CCS	Project 14 - Biomass	Project 15 - Co2Ras	Project 16 - Database	Project 17 - Expo	Project 18 Flexibag	Project 19 - Neptun	Project 20 - Particle	Project 21 - Photo_FW	Project 22 - Pocnad	Project 23 - Removal	Project 24 - Risk	Project 25 - Transfer	Project 26 - Treat	Project 27 - Preline	Sum

Costs (1000 NOK)

List of Post-docs, Candidates for PhD and MSc degrees during the full period of the centre

Post-doctoral researchers working on projects in the centre with financial support from other sources

Name	M/F	Nationality	Source of funding	Scientific area	Years in centre	Scientific topic	Main contact
Nhut Tran-Min	М	Vietnam	NFR	Detection technology	2	Fish health/POCNAD	Frank Karlsen
Nobotu Kaneko	М	Japan	NFR/JSPS	Fish physiology	2	OPTIMIZE/ROBUST	Tom Ole Nilsen
Shazia Aslam	F	France	NTNU	Marine Chemistry	3	Environmental chemistry	Øyvind Mikkelsen
Darragh Doyle	М	Irish	UGOT	Fish physiology	3	Skin barrier	Henrik Sundh
Junjie Zhang	М	Chinese	NTNU	RAS technology	4	Fish health in RAS	Alexandros Asimakopoulos

PhD candidates who have completed with financial support from the centre budget

Name	M/F	Nationality	Scientific area	Years in centre	Thesis title	Main thesis Advisor
Patrik Tang	М	Finnish	Post-smolt physiology	4	Thermal biological limits for osmoregulation, stress resilience and welfare in post-smolt Atlantic salmon (Salmo salar L.)	Sigurd Stefansson
Xiaoxue Zhang	F	Chinese	Sensor technology	4	The development of antifouling materials with potential application on sensors	Øyvind Mikkelsen
Patricia Aguilar Alarcon	F	Spanish	Water quality/- sensor technology	4	Deciphering the composition and transformation of dissolved organic matter in recirculating aquaculture systems by high- resolution mass spectrometry	Øyvind Mikkelsen
Sharada Navada	F	Indian	RAS technology	4	Salinity acclimation strategies for nitrifying bioreactors in recirculating aquaculture systems	Øyvind Mikkelsen

PhD candidates who have completed with other financial support, but associated with the centre

Name	M/F	Nationality	Source of funding	Scientific area	Years in centre	Thesis title	Main thesis Advisor
Sara Calabrese	F	Swedish	NFR/MOWI/ NORCE/UiB	Post-smolt physiology	3	Environmental and biological requirements of post-smolt Atlantic salmon (Salmo salar L.) in closed-containment aquaculture systems	Sigurd Handeland/ Cato Lyngøy
Lene Sveen	F	Norwegian	NFR/ NOFIMA	Post-smolt skin health	4	Aquaculture relevant stressors and their impacts on skin and wound healing in post- smolt Atlantic salmon (Salmo salar L.)	Elisabeth Ytterborg
Enrique Pino Martinez	М	Spanish	NORCE/ NFR/ KABIS	Post-smolt maturation	4	Influence of water temperature, photoperiod and feeding regime on early sexual maturation of Atlantic salmon (Salmo salar L.) postsmolts in freshwater	Sigurd Handeland
Bernat Morro	м	Spanish	RFF Vest	Trout physiology	4	The Physiology of Smoltification and Seawater Adaptation in Rainbow Trout	Simon MacKenzie, Univ. Stirling
Tharmini Kalananthan	F	Sri Lanka	RFF Vest/ UiB	Fish nutrition	4	Basic mechanisms for control of appetite and feed intake in Atlantic salmon (Salmo salar)	lvar Rønnestad
John Davidson	М	USA	NFR/ Freshwater Institute	RAS	4	Evaluating the suitability of RAS culture environment for rainbow trout and Atlantic salmon: A ten-year progression of applied research and technological advancements to optimize water quality and fish performance	Dr. philos.

Name	M/F	Nationality	Scientific area	Years in centre	Thesis topic	Main thesis Advisor
Victoria Røyseth	F	Norwegian	Fish health	3	Fish health (replaced by Even Mjølnerud)	Are Nylund
Even Mjølnerød	М	Norwegian	Fish health	4	Fish health	Are Nylund
Ingrid Naterstad Haugen	F	Norwegian	Water quality/- sensor technology	4+	Water quality/sensor technology	Øyvind Mikkelsen
Marius Takvam	М	Norwegian	Smolt physiology	2	Smolt/Post-smolt physiology	Tom Ole Nilsen
Gaute Helberg	М	Norwegian	Physiology/- aquaculture	2	RAS technology	Jelena Kolarevic
I-Hao Chen	М	Chinese	Aquaculture	2	Digitilazation in aquaculture	Naouel Gharbi
Wanhe Qi	М	Chinese	RAS technology	2	Water disinfection strategies to improve Atlantic salmon parr production in freshwater recirculating aquaculture systems (RAS-Health)	Lars Flemming Pedersen, DTU
Julie Hansen Bergstedt	F	Danish	RAS/Water quality	2	Prevalence and consequences of hydrogen sulphide in land-based Atlantic salmon production (H2Salar)	Peter Vilhelm Skov, DTU

PhD students with financial support from the centre budget who still are in the process of finishing studies

MSc candidates with thesis related to the centre research agenda and an advisor from the centre staff

Name	M/F	Nationality	Scientific area	Years in centre	Thesis title	Main thesis Advisor
Britt Sjöqvist	F	Swedish	Fish health	2	The Skin Barrier of Atlantic salmon (Salmo salar) - Characterisation and effects of husbandry related chronic and acute stress	Henrik Sundh
Ida Heden	F	Swedish	Fish health	2	Endocrine control and physiological mechanisms behind increased growth rate in Atlantic salmon (Salmo salar) reared in brackish water	Henrik Sundh/ Elisabeth Jönsson Bergmann
Øyvind Moe	м	Norwegian	Performance and welfare	2	The effect of aerobic training on growth, muscle development and heart condition in Atlantic salmon (Salmo salar) postsmolts in large-scale semi-closed containment systems.	Tom Ole Nilsen, Sigurd Handeland
Ingrid Gamlem	F	Norwegian	Performance and welfare	2	Documentation of growth and insulin-like growth factor I in Atlantic salmon (Salmo salar) post-smolts reared in large scale semi-closed and open systems	Tom Ole Nilsen, Sigurd Handeland
Egor Gaidukov	м	Ukraine	Performance and welfare	2	Effects of rapid temperature changes on Atlantic salmon, Salmo salar, post-smolts	Tom Ole Nilsen
Hilde Frotjold	F	Norwegian	Health	2	Diversitet, prevalens og tetthet av patogener i semi- lukkede anlegg (S-CCS)	Are Nylund
Thomas Kloster-Jensen	м	Norwegian	Health	2	Development of a genotyping tool for Salmonid Gill Poxvirus (SGPV) in farmed- and wild salmon (Salmo salar) in Norwegian waters	Are Nylund
Gunnar Berg	м	Norwegian	Performance and welfare	2	Neuroendocrine factors involved in appetite control and feed intake in Atlantic salmon (Salmo salar) reared in Recirculating Aquaculture Systems (RAS).	lvar Rønnestad
Tarald Kleppa Øvrebø	м	Norwegian	Performance and welfare	2	Growth performance and welfare of post-smolt (Salmo salar L.) reared in semi closed containment systems (S-CCS) – a comparative study	Sigurd Handeland
Sjur Øyen	м	Norwegian	Health	2	Benchmarking gut health in relation to diet in Atlantic salmon (Salmo salar) in seawater flowthrough systems	Karin Pittman
Hilde Lerøy	F	Norwegian	Genetics	2	Diploid og triploid Salmo salar, -og Salmo salar -Salmo trutta hybrider	Tom Ole Nilsen

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Name	M/F	Nationality	Scientific area	Years in centre	Thesis title	Main thesis Advisor
llona Nicolaysen	F	Norwegian	Stress and gut health	2	Stress responses and gut health of post-smolt Atlantic salmon (Salmo salar) exposed to chronic sub-lethal level of hydrogen sulphide in a recirculating aquaculture system	Carlo Lazado
Guro Berle Lokshall	F	Norwegwian	Smoltification in RAS	2	Effects of photoperiod regimes and salinity on Na+, K+-ATPase a-isoform expression in gills and kidney during smoltification in Atlantic salmon (Salmo salar) in recirculating aquaculture systems	Tom Ole Nilsen
Ross Fisher Cairnduff	м	British	Health and welfare	2	Mechanisms behind endocrine disruption of Smoltification and seawater tolerance in Atlantic Salmon (Salmo salar) by Testosterone, 11 Keto Testosterone and 17β-Estradiol	Tom Ole Nilsen
Marius Takvam	м	Norwegian	Performance and welfare	2	Development of Na+, K+ ATPase enzyme activity and expression patterns of sulfate transporters in gills, intestine and kidney during smoltification and SW acclimation in Atlantic salmon (Salmo salar L.)	Tom Ole Nilsen
Tilde Sørstrand Haugen	F	Norwegian	Performance and welfare	2	The effect of winter-signal photoperiod manipulations in smoltification of Atlantic salmon (Salmo salar) reared in commercial Recirculation Aquaculture Systems (RAS)	Sigurd Handeland
Markus Brånås	м	Norwegian	Performance and welfare	2	Influence of temperature and feeding on early sexual maturation commitment in male Atlantic salmon (Salmo salar, L.) during the freshwater stage	Sigurd Handeland
Bibbi Hjelle	F	Norwegian	Performance and welfare	2	Field study on Atlantic salmon (Salmo salar) acoustic delicing: Fish welfare and salmon lice (Lepeophtheirus salmonis) dynamics	Sigurd Handeland
Kristine Kannelønning	F	Norwegian	Performance and welfare	2	Parameterization of caged salmon feeding behaviour by investigating the relationship between fish density measured by fish counting and hydroacoustic observation	Ole Folkedal (HI), Tom Ole Nilsen
Kari Anne Kamlund	F	Norwegian	Performance and welfare	2	The effect of temperature and photoperiod on early sexual maturation in male Atlantic salmon (Salmo salar L.) during the freshwater phase	Sigurd Handeland
Trine Tangerås Hansen	F	Norwegian	Performance and welfare	2	The effects of water temperature and photoperiod: Investigating the cause of early activation of the BPG axis in male Atlantic salmon (Salmo salar) during the freshwater stages of development	Mitchell Fleming
Sigval Myren	м	Norwegian	Performance and welfare	2	Comparing the Na+/K+-ATPase enzyme activity of intestine, gills and kidney of Atlantic salmon Salmo salar during smoltification and exploring the effects of microplastic on intestinal function.	Tom Ole Nilsen
Ylva Mathilde Osdal	F	Norwegian	Health	2	Pathogen screening in Semi Closed Containment Systems and open net pens with emphasis on the presence of Tenacibaculum spp. and development of Real Time RTPCR assays for Tenacibaculum spp.	Are Nylund
August Sindre	м	Norwegian	Performance and welfare	2	Post-smolt performance of Atlantic salmon (Salmo salar L.) under strict photo regime before and after SW transfer	Tom Ole Nilsen
Siri Marie Lillebostad	F	Norwegian	Health	2	Phylogenetic and histological analysis of Candidatus Branchiomonas cysticola associated with epitheliocystis in farmed and wild salmonids in Norway	Are Nylund
Erik Heimdal	м	Norwegian	Performance and welfare	2	Different production protocols influence smolt physiology and growth in seawater for Atlantic salmon (Salmo salar)	Tom Ole Nilsen
Sofie Agnethe Isaksen	F	Norwegian	Health	2	Genetic variation of Piscine orthoreovirus and the presence of HSMI in farmed Atlantic salmon from Arctic Norway	Are Nylund
Steinar Bårdsnes	м	Norwegian	Water quality	2	Investigating potential effects of organic load and microparticle accumulation on light quality and quantity in recirculating aquaculture systems	Andre Meriac
Guro Berge Lokshall	F	Norwegian	Performance and welfare	2	The effect of time and length of light-induced smoltification in RAS for smolt quality and performance in SW	Tom Ole Nilsen

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Name	M/F	Nationality	Scientific area	Years in centre	Thesis title	Main thesis Advisor
Simen Haaland	М	Norwegian	Performance and welfare	2	Semi-closed containment systems in Atlantic salmon production - Comparative analysis of production strategies	Øyvind Mikkelsen
Lena Hovda Aas	F	Norwegian	Health	2	Dietary effects on growth, skin morphology and stress marker genes of Atlantic salmon (Salmo salar L.) smolts	Carlo Lazado
Danilo Carletto	М	Italian	Performance and welfare	2	Physiological hallmarks of Atlantic salmon parr responses to an oxidant	Carlo Lazado
Kamilla Grindedal	F	Norwegian	Water quality	2	Water quality in closed-containment aquaculture systems (CCS) for Atlantic salmon post-smolt - Non-target screening of organic substances using UPLC-MS/MS	Øyvind Mikkelsen
Maia Langøy Eggen	F	Norwegian	Health	2	Determination of peracetic acid (PAA) exposure concentration and administration methods on Atlantic salmon.	Carlo Lazado
Hanna Ross Alipio	F	Philippines	Health	2	Hydrogen sulphide as a regulator of mucosal immune defences in Atlantic salmon	Carlo Lazado
Guiseppe Scaduto	М	Italian	Health	2	Mucosal immune responses of Atlantic salmon following a simulated biosecurity breach in RAS	Carlo Lazado
llona Nicolaysen	F	Norwegian	Health	2	Impacts of long-term exposure to sub-lethal levels of hydrogen sulphide in Atlantic salmon reared in RAS,	Carlo Lazado
Bosco Ara Diaz	М	Portugese	Health	2	Immunological function of H2S both in the early life and juvenile stages of Atlantic salmon	Carlo Lazado
Bedika Ghising	F	Nepal	Health	2	Physiological functions of H2S in Atlantic salmon liver in vitro and in vivo	Carlo Lazado
Claudia Spanu	F	Spanish	Water quality	2	Osmotic stress priming as a microbial management strategy for improving salinity acclimation in nitrifying biofilms	Øyvind Mikkelsen
Nikko Alvin Cabillon	М	Philippines	Performance and welfare	2	Plasticity of muscle growth and mucosal defenses in Atlantic salmon (Salmo salar) subjected to different exercise intensities	Carlo Lazado
João Osório	М	Portuguese	Health	2	Mucosal and physiological responses of Atlantic salmon (Salmo salar) in brackish water RAS following peracetic acid-based disinfection	Carlo Lazado
Kristin Søiland	F	Norwegian	Water quality	2	Analysis of Ammonium and Nitrate with Ion Selective Electrodes (ISE) in Recirculating Aquaculture Systems (RAS) and a Test of Ag – TiO2 Nanotube Composite Material as Antifouling Sensor Protection.	Øyvind Mikkelsen
Caroline Berge Hansen	F	Norwegian	Water quality	2	In Situ Assessment of a Novel Underwater pCO2 Sensor Based on NDIR Spectrometry Enclosed in a Semipermeable Membrane for use in Aquaculture. Evaluation of Four Biofouling Protection Mechanisms.	Øyvind Mikkelsen
Nefeli Simopoulou	F	Greek	Health	2	Functional Characterization of the skin barrier of Atlantic wolffish (Anarhichas Lupus) and rainbow trout (Oncorhynchus Mykiss)	Henrik Sundh
Gisle Roel Bye	М	Norwegian	Water quality	2	Physio-chemical effects of different CO2 level in recirculating aquaculture systems (RAS) for Atlantic salmon post-smolt With focus on heavy metals	Øyvind Mikkelsen
Anusha Lamichhane	F	Nepal	Genetic	2	Genetic variation in egg traits and early survival in rainbow trout	Bjarne Gjerde
Kari Takvam Justad	F	Norwegian	Water quality	2	Atlantic salmon water pathogens inactivation by UV irradiation	Vasco Mota

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Name	M/F	Nationality	Scientific area	Years in centre	Thesis title	Main thesis Advisor
Julie Elise Trovåg	F	Norwegian	Health	2	Dietary effects on growth, skin morphology and stress marker genes of Atlantic salmon (Salmo salar L.) smolts	Christian René Karlsen
Marianna Sebastianpillai	F	Norwegian	Water quality	2	Start-up of freshwater and brackish water biofilm reactors in RAS: Nitrification activity and microbial community structure	Øyvind Mikkelsen
Bjørn Anda Estensen	м	Norwegian	Water quality	2	The impact of redox reactions on the N- and P-cycle in recirculating aquaculture system	Murat Van Ardelan
Clara Gansert	F	German	Water quality	2	Characterisation of changes in dissolved organic matter (DOM) composition of recirculating aquaculture system (RAS) sludge at different redox stages.	Murat Van Ardelan
Magne Bjørnstad Vrangen	м	Norwegian	Water quality	2	Nitrous oxide and hydrogen sulfide production in sludge from a recirculating aquaculture system (RAS)	Murat Van Ardelan
Yemima Tanudjaja	F	Indonesian	Water quality	2	Early Warning Signs and Dynamics of H2S Production In Recirculating Aquaculture System	Murat Van Ardelan
Ingrid Gjerde	F	Norwegian	Water quality	2	Study of water quality with regard to chemical parameters in RAS systems in aquaculture	Øyvind Mikkelsen
Rolf Klokkerengen	м	Norwegian	Health	2	Assessment of the oxidative stress biomarkers of 8-hydroxy-2'-deoxyguanosine and o,o'-dityrosine in skin and gill tissue from Atlantic salmon Parr in land-based recirculating aquaculture systems (RAS)	Øyvind Mikkelsen
Eivind Branzæg Sundfør	м	Norwegian	Health	2	Assessment of the oxidative stress biomarkers of 8-hydroxy-2'-deoxyguanosine and o,o'-dityrosine in dorsal fin and liver tissue from Atlantic salmon Parr in land- based recirculating aquaculture systems (RAS)	Øyvind Mikkelsen
Miguel Guerreiro	м	Portugese	Health	2	Effect of low-pressure and medium-pressure UV radiation on Atlantic salmon pathogens' inactivation	Vasco Mota
Anushree Mainali	F	Nepal	Health	2	Peracetic acid disinfection in freshwater recirculating aquaculture systems: comparison of consumption of peracetic acid in different sample systems	Vasco Mota
Samaneh Mousavi	F	Nepal	Health	2	Evaluation of ozone and peracetic acid use during a Yersinia ruckeri challenge in Atlantic salmon recirculating aquaculture systems	Vasco Mota

Peer-reviewed Publications

Peer-reviewed publications

- Alipio, H.R., Hansen-Bergstedt, J., Lazado, C.C. (2023). Differential sensitivity of mucosal organs to transient exposure to hydrogen sulphide in post-smolt Atlantic salmon (Salmo salar). Aquaculture, 739595.
- Karlsen, C., Ytteborg, E., Furevik, A., Sveen, L., Tunheim, S., Afanasyev, S., Tingbø, M.G., Krasnov, A. (2023). Moritella viscosa early infection and transcriptional responses of intraperitoneal vaccinated and unvaccinated Atlantic salmon. Aquaculture. 572 (2023) 739531.
- Lazado, C.C., Voldvik, V., Timmerhaus, G., Andersen, Ø. (2023). Fast and slow releasing sulphide donors engender distinct transcriptomic alterations in Atlantic salmon hepatocytes. Aquatic Toxicology. 260:106574.
- Lepine, C., Redman, N., Murray, M., Lazado, C.C., Johansen, L-H., Espmark, Å.M., Davidson, J., Good, C. (2023). Assessing Peracetic Acid Application Methodology and Impacts on Fluidized Sand Biofilter Performance. Aquaculture Research, vol. 2023, Article ID 6294325, 6 pages, 2023.
- Aguilar-Alarcon, P., Zherebker, A., Rubekina, A., Shirshin, E., Simonsen, M.A., Kolarevic, J., Lazado, C.C., Nikolaev, E.N., Asimakopoulos, A.G., Mikkelsen, Ø (2022). Impact of ozone treatment on dissolved organic matter in land-based recirculating aquaculture systems studied by Fourier transform ion cyclotron resonance mass spectrometry. . Total Environment. 843:157009.
- Alipio, H.R., Albaladejo-Riad, N., Lazado, C.C. (2022). Sulphide donors affect the expression of mucin and sulphide detoxification genes in the mucosal organs of Atlantic salmon (Salmo salar).. Frontiers in Physiology. 13:1083672.
- Bergstedt, J.H., Skov, P.V., Letelier-Gordo, C.O. (2022). Efficacy of H2O2 on the removal kinetics of H2S in saltwater aquaculture systems, and the role of O2 and NO3-. Water Research. Volume 222, 15 August 2022, 118892.
- Cabillon, N.A.R., Lazado, C.C (2022). Exogenous sulphide donors modify the gene expression patterns of Atlantic salmon nasal leukocytes. Fish & Shellfish Immunology. 120:1-10.
- Carletto, D., Breiland, M.W., Hytterød, S., Timmerhaus, G., Lazado, C.C. (2022). Recurrent oxidant treatment induces dysregulation in the brain of

transcriptome of Atlantic salmon (Salmo salar) smolts. Toxicology Reports. 9:1461-1471.

- Cui, W., Takahashi, E., Morro, B., Vigo, P.B., Albalat, A., Pinto Pedrosa, C., Mackenzie, S.A., Nilsen, T.O., Sveier, H., Ebbesson, L.O.E., Handeland, S.O., Shimizu, M. (2022). Changes in circulating insulinlike growth factor-1 and its binding proteins in yearling rainbow trout during spring under natural and manipulated photoperiods and their relationships with gill Na+, K+-ATPase and body size. Comparative Biochemistry and Physiology A. 268:111205.
- J Benktander, H Sundh, S Sharba, S Teneberg, SK Lindén (2022). Aeromonas salmonicida binds a2-6 linked sialic acid, which is absent among the glycosphingolipid repertoires from skin, gill, stomach, pyloric caecum, and intestine. Virulence. 13 (1), 1741-1751.
- K Sundell, GM Berge, B Ruyter, H Sundh (2022). Low Omega-3 Levels in the Diet Disturbs Intestinal Barrier and Transporting Functions of Atlantic Salmon Freshwater and Seawater Smolts. Frontiers in Physiology, 805.
- Karlsen, C., Tzimorotas, D., Robertsen, E.M., Kirste, K.H., Bogevig, A.S., Rud, I. (2022). Feed microbiome: confounding factor affecting fish gut microbiome studies. ISME COMMUN. 2, 14, 2022.
- Klykken, C., Reed, A.K., Dalum, A.S., Olsen, R.E., Moe, M.K., Attramadal, K., Boissonnot, L.J.E. (2022). Physiological changes observed in farmed Atlantic salmon (Salmo salar L.) with nephrocalcinosis. Aquaculture. 554.
- Lazado Carlo C., Stiller Kevin T., Shahzad Khurram, Reiten Britt Kristin M., Marchenko Yuriy, Gerwins Jascha, Radonjic Filip Strand, Eckel Bernhard, Berge Arne, Espmark Åsa Maria (2022). Health and Welfare of Atlantic Salmon in FishGLOBE V5 – a Novel Closed Containment System at Sea. Frontiers in Animal Science. Volume 3, June 27, 2022.
- Lazado, C.C., Espmark, Å.M., Freire, R. (2022). Biology meets technology: Aquatic animals in novel and new aquaculture production systems. . Frontiers in Animal Science. 3: 102126.
- Mjølnerød E.B., Srivstava, A., Moore, L.J., Plarre H., Nylund, A. (2022). Identification of housekeeping genes of Cndidatus Branchiomonas cysticola associated with epitheiocystis in Atlantic salmon (Salmo salar). Archives of Microbiology. 2022, 204;365.
- Mota, V.C., Brenne, H., Kojen M., Rivers

Marhaug, K., Jakobsen, M. (2022). Evaluation of an ultrafiltration membrane for the removal of fish viruses and bacteria in aquaculture water. Frontiers in Marine Science - Marine Fisheries, Aquaculture and Living Resources 9:1037017.

- Navada, S., Vadstein, O. (2022). Salinity Acclimation Strategies in Nitrifying Bioreactors. Frontiers in Marine Science.
- Ovrebo Kleppa, T., Balseiro, P., Stefansson, S. O., Tveteras, R., Sveier, H., Imsland, A. K. and S. O. Handeland (2022). Investigation of growth performance of post-smolt Atlantic salmon (Salmo salar L.) in semi closed containment system: A big-scale benchmark study. AQUACULTURE RESEARCH. Vol 53: 4178-4189.
- P.A. Tang, S.O. Stefansson, T.O. Nilsen, N. Gharbi, F. Lai, V. Tronci, P. Balseiro, M. Gorissen, L.O.E. Ebbesson, (2022). Exposure to cold temperatures differentially modulates neural plasticity and stress responses in post-smolt Atlantic salmon (Salmo salar). Aquaculture. Volume 560, 2022.
- Tang P. A., Gharbi N., Nilsen T. O., Gorissen M., Stefansson S. O., Ebbesson L. O. E. (2022). Increased Thermal Challenges Differentially Modulate Neural Plasticity and Stress Responses in Post-Smolt Atlantic Salmon (Salmo salar). Frontiers in Marine Science. Volume 9, 2022.
- Ytrestøyl, T., Hjelle, E., Takle, H., Kolarevic, J., Rebl, A., Afanasyev, S., Krasnov, A., Brunsvik, P., Terjesen, B.F. (2022). Photoperiod in recirculation aquaculture systems and timing of seawater transfer affect seawater growth performance of Atlantic salmon (Salmo salar). Journal of the World Aquaculture Society. 1– 23, 2022.
- Benktander J., Sundt H., Sundell, K., Mohana Murugan A.V., Venkatakrishnan V., Padra J.T., Kolarevic J., Terjesen B.F., Gorissen M., Lindén S.K. (2021). Stress impairs skin barrier function and induces α2-3 linked N-acetyl neuraminic acid and core 1 0-glycans on skin mucins in Atlantic salmon, Salmo salar . Int. J. Mol. Sci. 2021, 22(3), 1488.
- Lazado C. C., Stiller K. T., Megård Reiten M.K., Osório J., Kolarevic J., Johansen L.H. (2021). Consequences of continuous ozonation on the health and welfare of Atlantic salmon postsmolts in a brackish water recirculating aquaculture system. Aquatic Toxicology. Volume 238, September 2021, 105935.

Morro, B., Broughton, R., Vigo, P.B.,

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Handeland, S.O., Mackenzie, S.A., Doherty, M.K., Whitfield, P.D., Shimizu, M., Gorissen, M., Sveier, H., Albalat, A. (2021). Endoplasmic reticulum stress as a key mechanism in stunted growth of seawater rainbow trout (Oncorhynchus mykiss). BMC Genomics. 22:824.

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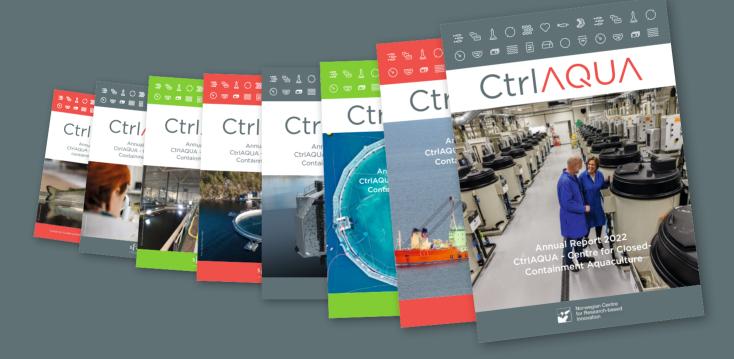
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