ANNUAL REPORT 2015
SEA LICE RESEARCH CENTRE
SUMMARY BY THE CENTRE DIRECTOR

In March 2015, SLRC staff, post docs, PhD-students and representatives from our partners met with the evaluation panel as a part of the mid-term evaluation (MTE). All participants were well prepared and the 3 meetings with the panel went very well. The conclusion from the MTE came from the Research Council late September and gave us a green light meaning that SLRC can continue for all 8 years without any new requirements from the Research Council. The report from the evaluation panel (MTE report) was very positive for SLRC and it was stated in the conclusion that we are an excellent and highly performing centre. This means that we have been able to establish a research centre that is performing well based on the evaluation criteria. However, there are also strong expectations that the SLRC delivers at a high level in the last 4 years of the centre life time and that the SLRC delivers new tools for sea lice control in close collaboration with our partners. One of the recommendations from the MTE panel was an increased focus on new medicine and vaccine development. Both are activities that require long term research efforts and as such fits well within a centre like the SLRC. These and other recommendations from the MTE panel have been implemented in the 2016 year plan.

2015 has been a challenging year in terms of sea lice control in many farms and the direct and indirect cost of lice control is increasing. An important reason is due to increased resistance to medicines. Efficient anti-sea lice medicines have been key tools in salmon louse control up to the present. Development of new medicines is a long term effort with high cost where a large proportion of tested and evaluated compounds will be dismissed before reaching commercial access. Scientists from the SLRC have identified and assessed several new targets in the salmon louse where new medicines will have a good effect. There are currently large efforts to develop non-medical tools for sea lice control and several different tools are under testing. However, most of these non-medical tools must be integrated with other control measures demanding that integrated pest management is further developed for sea lice.

Obtaining additional funding from different sources is expected for centres like the SLRC. SLRC scientists have been successful in obtaining more funding from various sources. These associated projects typically emerge as spin-off or extension of activity that is based on results or new research tools generated within the SLRC. In one of these associated projects salmon louse copepodids that can be identified genetically has been used to successfully challenge commercial size sea cages with Atlantic salmon. Data from this study showed that we are able to find off-springs of released lice on neighbour farms. Understanding how salmon lice are dispersed ("who infects who") is currently not well understood since the
origin of the lice on any given host is unknown. These novel findings may be used in the future to develop a tool to enhance our understanding about salmon louse dispersal in time and space, and serve as a valuable resource to validate models and lice infection strategies.

The funding from the Research Council to the SLRC ends in September 2019. One of the tasks for the MTE was to describe an exit strategy for the centre. Now that we know the outcome from the MTE, it is time to start the discussion about the SLRC future beyond the funding and SFI-status from the Research Council. The outcome of this is to a large extent based on interest from partner of the SLRC, and if they see a role for the SLRC beyond 2019.
BACKGROUND OF THE SEA LICE RESEARCH CENTRE – CENTRE FOR RESEARCH-BASED INNOVATION

Sea lice (Lepeophtheirus salmonis and Caligus spp.) are the major pathogens affecting the global salmon farming industry and have a significant impact in many areas. The annual loss has recently been estimated to €300 million and the aquaculture industry relies heavily on a few medicines for lice control. Emerging resistance development to these medicines increases the necessity to develop new treatment methods (biological, prophylactic and new medicine) and tools to avoid increased losses due to sea lice and to ensure a sustainable salmon farming industry in the future.

Research conducted at the centre will focus on methods and tools to facilitate the development of new medicines, develop new tools for resistance monitoring, reduce attachment in infective stages, improve host response, identification and evaluation of new targets for a future sea lice vaccine and to explore the possibilities to utilize RNAi as a novel method in lice control. By using the salmon louse genome sequence as a starting point, functional genomics methods will be utilized to identify molecular markers for drug resistance to facilitate monitoring and prolong the life time for valuable anti sea lice drugs. The Sea Lice Research Centre (SLRC) consists of the leading scientists within the field together with the major industrial players to represent a strong consortium to develop short and long term solutions for one of the most significant problems for the salmon farming industry world-wide. This will be achieved through state of the art research in relevant fields (parasitology, molecular biology and genomics, pharmacology, host parasite interactions) and establishment of an integrated database resource for the salmon louse genome in addition to state of the art wet-lab facilities for sea lice research. Results from the SLRC will enable an integrated control system to be established, based on key features in sea lice biology, to improve sustainability of the salmon farming industry.

CENTRE VISION AND GOALS

SLRC aims at becoming a world leader for research on the salmon louse and similar parasites. The nature of the centre will facilitate development of new methods for lice control and shorten the time from basic research to new products and tools for parasite control in the aquaculture sector to achieve a true integrated pest management in the future.

SLRC objectives
• New medicines and resistance monitoring & control methods (WP1)
• Anti-attachment diets (WP2)
• Immune controls (specific & non-specific) (WP3, WP4)
• RNAi gene techniques for research tool development and future controls (WP4)
• In depth knowledge of the molecular biology of growth, reproduction and endocrine systems in sea lice (WP4)
• Annotated genome sequence linked into an integrated database containing experimental data (WP5, Licebase)
• Updated microarray and other molecular tools (WP3, 4, and 5)
• Larval detection and assessment techniques (WP4)
• Sea lice facility (naive lice population, challenge facility, etc.) (WP6, LiceLab)
• Development of true integrated pest management techniques for industry (Part V)
SLRC PERSPECTIVES

The manifested threat from sea lice infestation must be controlled in order for the fish farming industry to continue a sustainable growth. The SLRC’s goal is to develop solutions to this challenge. We need environmentally safe, efficacious and lasting control measures. Sea lice cannot be eradicated and our response needs to be a combination of prevention and treatment methods put together in a diversified, dynamic Integrated Pest Management toolbox.

As the new chairman of the Board, I am thrilled by the enthusiasm, knowledge and impatience demonstrated by the researchers and the partners in the consortium. The establishment of the unique sea lice laboratory stands out internationally. Insight in the biology of sea lice is key to finding new ways in combatting lice. All the work packages (WPs) show good progress and provided unprecedented knowledge base to harvest from going forward.

Entering the second phase of the centre’s existence, after the successful midway review, puts pressure on continuous progress and further focus to achieve results that may be commercialised. There are results to be further explored within functional feeds, medicines, resistance, immunological and biological interference as well as non-medical treatment alternatives. The Board needs to do the difficult strategic prioritising between different initiatives going forward. The input from the fish farming partners is important to choose the right target areas.

An unparalleled research centre has been created since the establishment in 2011. As little evidence suggest a transient nature of the sea lice challenge, the Board will search to establish a future permanent operation of the centre beyond 2019. With the fundamental support from the Norwegian Research Council, the consortium includes high ranking academic institutions like the University of Bergen, hosting the centre, as well as major players within fish farming, feed and pharmaceutical industry. This provides an excellent backbone for further cooperation, with emphasis on applied research.

Integrated pest management (IPM) remains the solution going forward, not only with respect to sea lice but also having regard to connected challenges. The SLRC has established an IPM Task Force and expects results also from this initiative as well as from the different WPs. With the dedication and energy I observe in the management, amongst the researchers and between the partners – I strongly believe that the SLRC will fulfill its goals, provide value for the fish farming society and its players going forward and thus deserve a permanent operation.

Oslo/Bergen, 25.2.16
Benedicte H. Fossum
Chairman of the Board

ORGANISATION

The SLRC has both academic and industrial partners outside Bergen, and the main scientific activity in the centre is located in Bergen and Oslo. People working for the SLRC at IMR and UiB are co-located in the SLRC facilities at UiB at Marineholmen.

The industrial partners are complementary to each other and linked together by the academic partners. The 8 SLRC partners in 2015 are:

University of Bergen (UiB) is the host institution for SLRC. Senior scientists from three departments at the faculty of mathematics and natural sciences are the base for the research in the SLRC. Senior scientists within biology, molecular biology and bioinformatics use their knowledge in the SLRC. The main wet-lab activities take place at UiB, where lice strains are kept. PhDs and Postdocs are educated within the center.

The Norwegian University of Life Sciences (NMBU) is represented in the SLRC with senior researchers from the Department of Food Safety and Infection Biology and the Department of Basic Sciences and Aquatic Medicine. PhDs and Postdocs are hired to work with the research in the SLRC. This partner is responsible for WP1 and WP3 and has close connections to WP2 and WP4. Until now, the main cooperating partners have been PatoGen Analyse, Elanco, EWOS and UiB.

Institute of Marine Research is represented in the SLRC with one senior researcher (80%), one post doc, one PhD student and one wet lab technician (50%). Major wet-lab activities, mainly RNAi trial takes place in the laboratories in Bergen. The Post doc and the PhD work in WP4, whereas the researcher works both in WP4 and WP6, where the technician also is connected.

EWOS Innovation AS is a user Partner in the SLRC with a long history of sea lice research. Scientists are based in Bergen and Dirdal where research facilities have been expanded the last years. In the SLRC, development of compounds that reduce the settlement and survival of lice will be the focus. EWOS Innovation is the leader of WP2 and is cooperating with NMBU in WP3 and UiB in WP6.

Lerøy Seafood Group ASA is one of the world leading salmon farming companies with more than 100 farming licenses in Norway, in addition to slaughterhouses and processing factories. In the SLRC, the company is a supplier of raw materials and facilities for field trials. Lerøy has also been an important contributor in the field validation of the novel analyses of the diagnostic PCR-analyses for resistance monitoring developed by PatoGen. First-hand information on needs and demands from the industry gives Lerøy an important role in the SLRC.

PatoGen Analyse AS is a biotechnology company that develops and sells gene technology analyses that are used to reduce disease related loss in the aquaculture industry. PatoGen has the most modern laboratories for Real-Time PCR analyses for detecting fish pathogens in
Norway, and work in close collaboration with research partners and industrial partners in the SLRC. PatoGen is mainly involved in WP1 and WP4, and collaborate with the partners UiB, NMBU, Marine Harvest ASA, Lerøy Seafood Group ASA and Elanco Animal Health.

Elanco Animal Health is a global company developing and commercializing leading animal treatments that meet the needs of pet owners, farmers and veterinarians; the company is fully owned by Eli Lilly&Company. Elanco Animal Health became a partner in SLRC following the acquisition of Novartis Animal Health AG by Eli Lilly&Company in January 2015. Elanco is involved in SLRC work through their Research & Development sites for vaccines (Prince Edward Island, Canada) and parasitology (Basel, Switzerland).

Marine Harvest ASA is a world leading seafood company, and is involved in all major salmon farming regions. The knowledge and international network is clearly an added value for the centre. Marine Harvest ASA has been an important contributor in the field validation of the novel analyses of the diagnostic PCR-analyses for resistance monitoring developed by PatoGen Analyse. In addition, Marine Harvest ASA is a supplier of raw materials and facilities for field trials in the SLRC.

 MANAGEMENT

UiB is as host for the SLRC responsible for the coordination of all activities in the centre. The day to day management is carried out at UiB by the Centre Director Frank Nilsen and the administrative coordinator Ingunn Wergeland.

The overall decision making body is the SLRC board, where all the partners have one representative each.

The Board takes decisions on strategy, annual work plans, activities, budget and the organisation. The SLRC board is chaired by an independent board leader. During 2015 there have been several replacements in the Board.

SLRC Board Members in 2015:
- Bjarne Reinert/Harald Sveier, Lerøy Seafood Group ASA
- Ragna Heggebø, EWOS Innovation AS
- Neil Robertson, Elanco Animal Health
- Gordon Ritchie/Olav Breck, Marine Harvest ASA
- Vidar Aspehaug, PatoGen Analyse AS
- Karin Kroom Boxaspen, Institute of Marine Research
- Lise Øvreås, University of Bergen
- Mona Aleksandersen, Norwegian University of Life Sciences
- Benedicte Fossum/Audun Wilborg – Chair of the board
CENTRE ACTIVITIES IN 2015

Two workshops for the SLRC personnel and partners have been arranged during the year:
5–6 May at Kringler Gjestegård with participation from most of the partners. The main purpose for the workshop was updating on the research going on in the various parts of the centre and to further develop and identify areas for collaboration between WPs and partners.

PhD-students and postdocs presented the scientific work carried out in the various WPs of the SLRC. To share knowledge is an important tool for securing the dynamic work in the SLRC and this meeting is an important facilitator to update partners on scientific progress.

The Scientific Advisory Board for the SLRC was invited to the Workshop both to meet all the centre staff but also to have meetings with the WP-leaders and board members in the SLRC. The annual “Spring Workshop” is a good opportunity for the SAB to meet all levels of the centre and to give a direct feedback on the research directions.

10–11 November at UiB: The workshop was organized in collaboration with Ensembl to introduce SLRC users to the Ensembl infrastructure and online tools. Members of the Ensembl training team gave a one day training course for the SLRC and possibilities for follow up on access to their tools. In addition, some of the upcoming PhD theses were presented as well as discussion on future direction for the centre.

COLLABORATION BETWEEN THE CENTRE’S PARTNERS

Combining the partners’ knowledge and expertise is a key factor to achieve the goals for the SLRC.

Research in the different WPs and sub-projects involves all the partners in the centre, and is an important tool to secure transfer of knowledge for innovation and development of new products and methodology. In 2015 the partners have prepared for the Mid Term Evaluation of the centre, and as a spin-off from planning meetings and future plans for the centre, the process has been a basis for new ideas and areas for further collaboration.

The partners have participated in workshops and meetings and used the possibilities for discussions at all levels in the organisation. New joint projects have been discussed and established – some of them with external funding and partners. Several of the projects are initiated by the industrial partners in the SLRC, which is strengthening the centre by involving other companies and organisations in associated projects.

One of the Postdocs at NMBU (WP1) has visited the Elanco lab in St. Aubin for 4 weeks in 2015. This exchange of personnel is strengthening the collaboration in the centre and is an important tool to speed up the possibility for new products development.

Visit to Elanco Animal Health, Switzerland
Kiran Kaur, Postdoc at NMBU

Elanco is one of the industry partners of the SLRC. Our group at the Pharmacology and toxciology department, Norwegian university of life sciences, works in close association with Elanco on various collaborative projects under the SLRC.

In summer 2015, I spent a fantastic week at Elanco’s headquarters in St. Aubin, Switzerland. This stay was a reward for winning the Elanco Young Scientist of the year title at 10th International sea lice conference in Portland, USA in 2014. The reward gave me a wonderful opportunity to have a great exchange of ideas with Elanco’s multi-disciplined team. The previous close collaborations, being a part of the SLRC, were quite advantageous to have known each other’s goal, but the week’s stay gave me the opportunity to present my work to a variety of groups and get their objective thoughts, which I’m sure, will benefit my future research. Moreover, I got the chance to witness, first hand, how the parasitology research programs are conducted at Elanco, and to observe the bio-analytic protocols and technologies they have developed.

This unique experience gave me an impression that we share the same passion for work, not only in the laboratory, but also for the practical outcomes that may be achieved in the field as a result of our science. Hence, I sincerely hope that we can stay in touch, which luckily is possible because of the SLRC, and hopefully take our collaborations further that will improve the management of sea lice infestations on salmon farms.

In the autumn 2015, the SLRC arranged a one-week course in sea lice biology. The main target group was Master and PhD students both from Norway and internationally. The course consisted of a mixture of lectures, student presentations and laboratory exercises. The lecturers presented data from a wide range of salmon louse research and included scientists from UiB, NMBU, IMR and Fisheries and Oceans Canada (DFO)/University of Victoria. In addition to the scientific activities, the students were also given the opportunity to get to know each other and the lecturers at a social event in the evening. Students from Norway, Portugal and Chile participated at the course.
During the Mid-term Evaluation process, members of the committee voiced the recommendation to train PhD students in the analysis of modern high-throughput data. With the implementation of a user-friendly pipeline for RNA-seq analysis in NeLS/Galaxy and in collaboration with Elixir.no, we have laid the foundation to do this. SLRC users have been trained in using NeLS and Galaxy in two workshops using real data.

In connection to the preparation of the 2016 work plan and frames for the IPM work in the centre, two meetings between the WP-leaders and industrial partners have been arranged.

3 Board Meetings have been arranged in 2015, hosted by IMR, EWOS and UiB.

The sea lice situation in Norway, seen from a farmer’s perspective
Bjarne Reinert, Fish Health Manager, Lerøy Seafood Group ASA

In terms of reported average levels of adult female sea lice, 2015 stands out as one of the years with lowest incidents of adult female sea lice. Regardless of the generally low reported levels, there have been large regional variations and the impact has been significant in many areas. Therapeutic resistance is still advancing and is now considered as a common situation along most of the coastline. As a consequence the efforts made to control infestations is tremendous and the cost associated with sea lice management is high.

Gradually reduced sensitivity against existing therapeutics has increased development of non-medical treatment alternatives. Freshwater, temperated-water, and different mechanical approaches are now frequently used and may be capable of reducing the industry’s reliance on existing pesticides. Although the efficacy of these non-medical treatment approaches is promising, they involve handling and should be used by caution. The overall target would be to maintain low average infestation levels without the need of treatments. Prevention is the key to future sea lice control and by only using treatments as a last bastion when prevention fails.

On the basis that no single measure is shown to be sufficiently effective on their own, farms utilize several different preventive measures as an integrated part of their sea lice management strategy. Cleanerfish, both farmed and wild-caught, is commonly used and considered as one of the most promising tools to maintain low average levels. This is a priority area for Lerøy Seafood Group, and by building competence and production capacity, we have great expectations that cleanerfish will play a crucial role in the ongoing fight against sea lice. Even though the efficacy seems promising on low average fish weights, the utilization of these species is in an early phase with regard to achieve their full potential.

Disregarded existing tools, there is a great need for comprehensive, and cutting edge research to facilitate future sea lice control. As an industrial partner, Lerøy Seafood Group is involved in the SLRC and believes the center would play a crucial role by generating valuable knowledge and tools to be adopted in an integrated manner.

Sea lice are sensitive to chemicals with different modes of action
Novel treatments towards sea lice can be found among molecules with proposed actions on several biochemical pathways. The nicotinic acetylcholine receptor, ecdysone receptors, various ion channels and acetyl coenzyme A have been identified as possible new targets for medicinal intervention. Because of widespread resistance towards the currently used treatments, the development of new treatments to be used as supplements to non-medical control will be important for future control of lice.

The results demonstrate that novel salmon louse medicines (for both bath and in feed treatments) with high effect can be developed. However, the time for when such new medicine can be commercial available depends on many factors and it is still years into the future.

SCIENTIFIC ACTIVITIES AND RESULTS

Work Package 1: Chemotherapy and resistance
Principle Investigator: Tor Einar Horsberg, NMBU

Medicinal treatment has been the most important control measure for sea lice and there is a strong need to develop new medicines and to understand why the old medicines are not working anymore.

This work package has two parts:

1. Explore possible new treatment methods and chemicals
The last molecule introduced as an antiparasitic agent for sea lice in Norway was emamectin benzoate (Slice™) in 1999. Due to the increasing problem with resistant salmon lice along the entire Norwegian coast, there is an urgent need to find new control options. The main aim of this project is to identify potential candidates that later can be developed into new treatments in cooperation with the partner from the pharmaceutical industry and others.

2. Develop robust assay methods for resistance testing against chemotherapeutants
Resistance against salmon lice treatment agents has been reported in Norway since 1991, but the problem has escalated dramatically since 2008. A crucial factor for effective management of resistance is reliable diagnostic methods. The main aim of this project is to identify the different resistance mechanisms and to develop rapid laboratory-based assays for these, in co-operation with the partner from the biotech industry and others.

LiceBase (WP5) has been an important tool for WP 1 in 2015. To obtain good material for the various studies, access to a seawater wet-lab has been rented from NIVA at the location Solbergstrand, Drøbak, 40 km from Oslo.
Explore possible new treatment methods and chemicals
During 2012–2014, standardized protocols for *in vitro* testing of efficacy of various chemicals on hatching of egg-strings, molting and direct toxic effects on motile parasites were developed. These have subsequently been used to study the *in vitro* effect of 27 chemicals from different MOA-classes on salmon lice. These “model substances” were selected in co-operation with Elanco, according to the IRAC mode of action classification (www.irac-online.org/teams/mode-of-action). The hatching- and nauplius part of the study was conducted in co-operation with UiB.

The studies were completed in 2015, and the results are currently in press. The studies demonstrated that very few compounds had a noticeable effect on the hatching of egg-strings in concentrations relevant for practical treatments. The exception was hydrogen peroxide, which inhibited hatching even at concentrations lower than 1/3 of the treatment concentrations. A possible explanation is the relative low permeability of the outer egg-string membrane to many chemicals.

Direct exposure of nauplii resulted in responses that could be divided into three distinct categories: 1) no significant effect; 2) a directly lethal effect; 3) an effect on development of nauplii into copepodides. In general, compounds with a direct, lethal effect on preadult parasites also had direct lethal effects on nauplii, but at different concentrations. Typical Kaplan-Meyer survival plots for these effects are presented in Figure 1.1. Exposure of preadult parasites revealed a number of compounds with a possible effect on salmon lice. The parasites displayed a considerable sensitivity towards compounds acting on the nicotinergic acetylcholine receptor, glutamate- and GABA-gated chloride channels, acetyl coenzyme A, and some juvenile hormone analogues.

The models are now used to screen through several experimental compounds supplied by Elanco.

To get more knowledge of the actual binding sites on the target proteins, some of these were modelled in 3D and subjected to *in silico* docking studies with several of the compounds. In figure 1.2, the predicted binding site for imidacloprid, a neonicotinoid, on the alpha subunit of the nicotinergic acetylcholine receptor is displayed. The predicted binding energy was compared with results from bioassays on preadult parasites. The agreement between predicted and observed results was good.

![Figure 1.1: Kaplan-Meyer survival plot of salmon lice exposed to chemicals in the nauplius phase. Panel 1 shows a typical plot for compounds with no noticeable effect (amitraz), panel 2 is a typical plot of compounds with a direct lethal effect on the larvae (azamethiphos), while panel 3 shows a typical plot for compounds inhibiting the development of the larvae (fenoxycarb). The control group is displayed in blue, the exposed group in red.](image)

![Figure 1.2: Predicted binding site for the neonicotinoid imidacloprid on the alpha-unit of the nicotinergic acetylcholine receptor in salmon lice.](image)

In 2014, a new project within WP 1 was initiated. The aim was to develop *in vitro* cultivation methods for developmental stages of the parasite that so far only can be cultivated using live fish. Initial results demonstrated that copepodides were able to develop frontal filaments on artificial substrates, but molting to chalimus stages could not be seen. Similarly, attempts to initiate molting from preadult I to preadult II were unsuccessful.
Develop robust assay methods for resistance testing against chemotherapeutants

During 2012–2014, a mutation causing resistance towards the organophosphate azamethiphos was detected, validated and patented together with the partner PatoGen Analyse AS (patogen.no/kontakt-oss/det-virker/licence-to-kill). In 2015, an extensive survey of this mutation in salmon lice sampled from the whole Norwegian coast was conducted by NMBU, PatoGen Analyse and the Norwegian Veterinary Institute. This study demonstrated the resistance alleles to be widespread. The highest frequency was found in Nord-Trøndelag, with high frequencies in northern Nordland and southern Hordaland as well. The lowest frequencies were found in Agder in the far south and Finnmark in the far north. The study is in press. In Figure 3, the weighed treatment intensities with azamethiphos are displayed in the left panel, while the distribution of resistant alleles is displayed in the right panel.

Figure 3: Maps showing relative quantities of the density of treatments (kernel density), along with the geoidex line (left panel). The proportion of the three genotypes RR (homozygote resistant, red), SS (homozygote sensitive, green) and RS (heterozygote, yellow) by farm is shown by pie charts in the right panel. Note that the location of farms is not exact due to space conflicts in the map.

Resistance towards sea lice treatments is widespread

Resistance towards practically all treatments against sea lice has been demonstrated in Norway. The situation is grave, and in some areas, no clinically significant effect can be expected from emamectin-benzoate, deltamethrin, cypermethrin, azamethiphos or hydrogen peroxide. A valuable tool for surveillance and control of resistance is molecular assays detecting the actual resistance mechanism at the genetic level. Such methods have the advantage that the dispersal of resistance genes can be monitored much more detailed compared to monitoring with biological methods (bioassays). Efforts have been made to identify resistance mechanisms for pyrethroid-, emamectin- and hydrogen peroxide resistance. Through a crossing experiment between sensitive and resistant salmon lice, both susceptible and resistant parasites from the F2 generation after these crossings were selected using discriminating doses of each compound. The samples have been submitted for RNAseq, and the results will be ready in April 2016. Material for subsequent qPCR of interesting genes has been secured from the same batches of parasites. In addition, phenotypic studies of the strains have been conducted. These involve bioassays, enzymatic assays and electrophysiological assays. The electrophysiological studies are conducted in collaboration with Elanco. These studies are ongoing.

In 2014, an associated project dealing with resistance in the Chilean parasite Caligus rogercresseyi was initiated. The studies were conducted in collaboration with Universidad de Austral, Puerto Montt, Chile and Cermaq Chile. A number of samples were collected in seven Chilean salmon farms and subjected to selection with azamethiphos, deltamethrin, emamectin benzoate and hydrogen peroxide. Azamethiphos and pyrethroids (deltamethrin and cypermethrin) were the most commonly applied treatments. Sensitivity towards azamethiphos was lower in Region X than in XI. Yet the treatment efficacy of azamethiphos treatments was above 90% in all farms. Sensitivity towards deltamethrin varied among farms; more resistance was found in the mid-west of Region X. Consequently, pyrethroid treatment efficacy was low in that location (39-79%). Deltamethrin sensitivity was negatively related with the number of treatments applied and positively related with pyrethroid treatment efficacies. The build-up of the louse abundance after a treatment varied between farms and anti-louse treatments, being considerably faster in the farm located in mid-west of Region X for deltamethrin. Sensitivity towards emamectin benzoate was low. The results from these studies are currently in press.

Deltamethrin-selected samples have been submitted for RNAseq studies and will be analysed in co-operation with UiB, WP5. The acetylcholin esterase gene in C.rogercressey has been fully sequenced in 2015, and several SNPs have been identified in this gene. These are currently examined for possible amino acid changes and whether or not they are associated with reduced efficacy of azamethiphos on this species.

Figure 4: Rainbow trout heavily infested with Caligus rogercresseyi. Photo: Sandra Bravo.
Scientific activities and results

Work Package 2: Anti-attachment

Principle Investigator: Simon Wadsworth, EWOS Innovation AS

Functional feed concept based on the inclusion of plant ingredients that possess anti-parasitic bioactive properties holds great promise in the control of sea lice. The development of anti-lice functional feeds would enable modulation of endogenous protective responses in fish; this approach is favored as it is administered without handling stress and is easily integrated into management practices, is active against multiple life stages, and is not likely to result in resistance development.

Sea lice are highly host-specific parasites
Sea lice such as *Lepeophtheirus salmonis* are only able to complete their life cycle on a very narrow range of salmonid species, where they are able to defeat the immune system of these hosts. If the lice attempt attachment onto non-host species, the immune response from these fish will be lethal to the parasite. Thus, correct identification of the salmon host is a matter of life and death for the lice.

Sea lice have advanced sensory systems (olfactory and contact chemoreceptors) that are capable of accurate identification of the salmon host molecules. These sophisticated receptors have recently been characterized by the Sea Lice Research Centre (SLRC).

Recent research has shown that host recognition can be masked by a number of compounds, resulting in a disruption of the host-identification and attachment processes for the lice. In turn, decreased immunomodulation by lice allows for a stronger, protective response from the host.

This work package has two main parts: *In vitro* methods and assay techniques and *In vivo* assessment of masking compounds in-feed and several sub projects described below.

**In vitro methods and assay techniques**

In previous reporting the Y-tube and Frontal Filament model were described (SLRC 2014). Briefly, the vertical Y-tube assay was used to study sea lice copepodid directional response (rheotaxis) and swimming activity to host odors. Once positive, consistent directional responses of the sea lice were observed to the salmon entrained water, a series of masking compounds were assessed on the activity. Significant inhibition was observed to the direction and activity of sea lice to a range of vegetable-based compounds. We have also seen decreased success of copepodid attachment in the presence of host odour combined with masking compounds, or in the absence of host odour.

An additional *in vitro* model was established using a solidified substrate of salmon mucus, mixed with agar. A thin layer of seawater was used to cover the substrate before copepodid stages of sea lice were added. Sea lice (*C. rogercressey*) were able to detect the salmon host odour within the substrate and quickly deployed their frontal filament. In the absence of salmon host odour, or in the presence of host odour combined with masking compounds, the sea lice declined to deploy their frontal filament. Using this method, the most effective plant-based products previously identified in the Y-tube assay have been validated as potential host masking compounds. The frontal filament model has been less successful for *L. salmonis*, as these lice require a number of days (up to 8) for the filament to develop. Maintaining a viable culture for more than 48 hours has proven challenging and has resulted in the loss of the *L. salmonis* before they were able to fully deploy their frontal filament. No further progress was made with *L. salmonis* during 2015 despite further attempts. However, an *in vitro* model (termed Mucus model) based on the screening of gene expression responses in copepodids simultaneously incubated for 24 hours with the Atlantic salmon mucus and masking compounds in glass containers with no substrate for attachment turned out to be feasible and more informative.

The Y-tube, Frontal Filament and Mucus model models only assess the effect of compounds directly exposed to sea lice. During 2015, two compounds that showed promise in these *in vitro* systems were taken through to large scale in-feed testing, using *in vivo* challenges on fish. No observable protection was observed either due to the absence of effects on endogenous defence mechanisms of the host or the metabolic degradation of tested compounds and lack of their expression in the mucus and skin of the fish. Further methods were required to determine effects on sea lice from compounds that had been expressed in fish tissues.

Development of the *Ex-vivo* fin tissue attachment model during 2015 was a step closer to *in vivo* studies. Sections of pectoral fins were removed from fish that had been fed either anti-attachment or control diets for a period of 14 days. The fin from control and test diet fed-fish were suspended and exposed to around 300 copepodids of *L. salmonis* (Figure 1). After 24 hours the numbers of lice attached to fin from control fed fish, fin from anti-attachment fed fish as well as free-swimming lice were assessed. The *Ex-vivo* fin tests were established with up to 10 replicates and repeated 7 times. Results showed a significant reduction in the number of lice settling onto the fins from fish fed the anti-attachment compounds. We have also seen decreased success of copepodid attachment in the *Ex-vivo* model under the exposure of 2’aminooacetophenon (2’-AA), the putative anti-lice molecule expressed in the turbot skin; and to the bioactive molecule found in the raw ingredient of the anti-attachment feed extensively tested since 2012. Further, we observed the effects of dose and pre-incubation time with anti-lice compounds on the success rate of attachment.

Profiling gene expression responses of *L. salmonis* copepodids from the *Ex-vivo* model under exposure of the anti-lice bioactive compound from feed revealed increased expression of genes coding for proteases potentially involved in moulting, digestion or as viral factors. Thus, the *Ex-vivo* model proved valuable in understanding the biology of attachment and early settlement of *L. salmonis* and will be incorporated into the screening system to validate effects of the *in vitro* models and provide further evidence of the successful dietary preconditioning of skin and fin tissues prior to *in vivo* testing.
In vivo assessment of masking compounds in-feed

- Assessment of masking compound on reducing lice numbers post challenge
- Assess combinations of masking compounds
- Determine fish gene responses during challenge (physiological and immune pathways)
- Determine lice gene responses exposed to functional components during challenge

Sea lice suppress and divert the immune response of salmon

Once sea lice have positively identified the correct host they release a range of factors that are highly effective at modulating the immune system of the host. As well as suppressing overall immunity, the lice are also able to divert the remaining responses away from focused and protective inflammatory Type 1 reactions. These effects on immunity are thought to significantly increase susceptibility of Atlantic salmon to sea lice infection. Maintaining overall immunity and more effective pathways represents a significant development in sea lice control. More recently, it has also been postulated that differences in iron and haem availability have significant effects on susceptibility to lice. Protection against sea lice in resistant Pacific species is apparently greatly affected by the ability of the host to restrict iron and haem availability to the parasite.

Two additional novel feed additives tested in in vivo challenge trials have not produced positive results during 2015. In addition, different sources of raw ingredients with established efficacy were tested for the dose effect and in combination with immunostimulants known to induce moderate protection against lice. However, no additive effects were observed in these studies either.

Certain Pacific species of salmon such as pink and Coho are highly resistant to sea lice and have a range of effective defenses that are able to kill all attached stages of lice within 21 days of infection. The SLRC has been working with leading research partners to gain a better understanding of these mechanisms, and to see if they can be enhanced by nutritional means in Atlantic salmon.

WP2 deployed microarray profiling of Atlantic salmon skin to learn more about the protection against salmon lice mediated by the anti-attachment feed. The analysis showed that the tested feed activated multiple genes of innate antiviral immunity and chemokines prior to lice challenge, suggesting a preconditioning effect. Moreover, their higher expression was maintained by the time lice reached the pre-adult stage. A number of candidates of protection emerged from this study, including lectins, antimicrobial peptide cathelicidin, extracellular matrix remodeling metalloproteinases and many novel Type 1 pro-inflammatory mediators. A few potentially immunopathological Type 1 effectors showed context-dependent regulation being down-regulated by feed before the lice challenge and enhanced upon infection (myeloperoxidase and cytokines IL-17 and IL-8). Exposure to the anti-attachment diet also changed expression of genes that can improve resistance by affecting the skin composition in infected fish.

WP2 also aimed to increase our understanding of the host mechanisms at earlier time points (4 and 8 days post-infection, as compared to 21-30 days post-infection studied previously). The gene expression analysis of head kidney, thymus and two different places from the skin showed that the default response to lice includes elements of Type 2 inflammatory reaction in all organs. In addition, histological techniques, including immunohistochemistry and transmission electron microscopy were used to understand proliferative processes in the skin and to identify different subpopulations of macrophages.

If key nutrients, such as iron are reduced in the skin and plasma of the fish, lice development can be delayed and the lice are then much more susceptible to the immune response from the host. Resistant species such as pink and coho salmon show up-regulation of a range of mechanisms that restrict iron in the skin and plasma during lice infection. One of the most important control factors includes hepcidin, a peptide hormone that reduces dietary iron absorption across the gut mucosa, as well as the release from macrophages (a major storage site). Lice are especially affected by limiting their access to iron. It has been recently indicated that lice are unable to produce haem and have to gain this from their host. Haem is an essential iron containing cofactor that is necessary for oxygen transport as part of hemoglobin. Many proteins involved in other critical functions have haem as their prosthetic group as well. When resistant salmon up-regulate hepcidin, there is a dramatic reduction in the availability of haem for the lice to utilise. Our work with the Chilean collaborator revealed that genes involved in the acquisition of iron become up-regulated in *C. rogeri* in response to our anti-attachment feed.

Our detailed expression profiling of Atlantic salmon responses to *L. salmonis* exposed to the anti-attachment feed revealed regulation of genes with roles in iron metabolism and activation of detoxification metabolism genes in the liver, distal kidney and muscle. The most highly induced gene in muscle was a potent antioxidant heme oxygenase-1 that degrades iron-containing heme. Hepatic down-regulation of porphobilinogen deaminase involved in heme biosynthesis and induction of hepcidin-1 also implied reduced iron level in blood. Finally, greater iron sequestration in response to the feed was suggested by the increased renal gene expression of ferritin, encoding a potent cytoprotective antioxidant.

Assessment of the functional feed programme at commercial sites has been initiated in Chile at several locations within 2015. Gradual reduction in lice counts were observed over a 4-month period; a follow up RNA sequencing profiling of salmon tissues by the Chilean collaborator (Gallardo et al; University of Concepcion) implicated activation of protective mechanisms seen by microarray profiling in Atlantic salmon infected with *L. salmonis* that was exposed to the anti-lice functional feed.

During 2015 a lot has been learned about the complexity of the olfactory system of *C. rogeri*, and the possibilities to modulate the involved ionotropic receptor genes by in-feed plant derived anti-lice bioactive additives. In order to investigate the genome-wide effects of the anti-attachment feed on *L. salmonis* we performed an RNA sequencing study in the lab of the Chilean collaborator (Gallardo et al., Uni of Concepcion), previously involved in the work on ionotropic receptor genes. Males in the pre-adult 2 stage were starved for 6 days and were then placed on fish fed control and test diets. Lice were left to feed for a day and then harvested for analysis. The RNAseq data revealed relevant differences between starved and fed lice; further analysis is ongoing.
Work Package 3: Immunomodulation of the host
Principal Investigator: Øystein Evensen, NMBU

WP3 addresses Immunomodulation of the host. The concept is that sea lice releases a series of secretory/excretory products (SEP) into the host tissue, via salivary glands to prevent strong inflammatory responses to infection. To better understand and design therapeutic intervention that can alleviate or counteract the effects of the secretory products, the underlying mechanisms of inflammation and anti-inflammatory processes must be understood.

Sea lice modulate the immune response of the host
Sea lice release chemical compounds upon attachment of the host that modulates the immune responses of the host. Modulation means down-regulation of diversion of host responses that are less deleterious to the sea lice and will facilitate attachment, development, moulting and production of progeny.

These mechanisms are only partly understood and more detailed deciphering of the host-pathogen cross-talk can provide clues to institute strategies through for example feed additives that can strengthen the host immune system and make them less prone to unfavourable sea-lice induced immunomodulation.

The two projects in WP3 “Evaluate in vitro the immunomodulatory effects of PGE-2 in a cell line derived from Atlantic salmon (TO cells)” and “Establish a method to identify proteins/components involved in host-parasite interaction, using a combined genomic / proteomic approach” were finalized in 2015 and will not be explored any further in the coming years. Summarizing, we have studied the effect of PGE2 on Atlantic salmon-derived cell lines (first project) where the effects of PGE2 on immune dampening was examined. Over the last two years, the work has mainly devoted to documenting these findings and pursuant to this, a manuscript was published describing tissue distribution of EP4 receptors in Atlantic salmon (Gamil et al. 2015). No further studies will be pursued to describe PGE2 effects in vitro.

For the second project, results are published (Guo et al. 2015) which documents the EP4 receptor sequence and presence of two isoforms in Atlantic salmon. The phylogenetic relationship with other species is shown in Figure 3.2.

The main activity in WP3 for 2015 has been divided into two projects:

In vivo fish (Atlantic salmon) challenge with copepodids. – Assessment and characterization of local responses to infection including cellular components and mediators (cytokines/chemokines) and transcript profiling of local responses to infection.

Studies on the local inflammatory skin responses were conducted in collaboration with WP2 (EWOS) and these results were published in 2015 (Holm et al. 2015). Fish from families with high genetic resistance have a response biased towards a Th1 cytokine profile and thus carry lower number of sea lice, while the default response in less resistant fish has a Th2 bias.

Figure 3.1: The expression of asEP4 receptor in different tissues by PCR. Real-time PCR was performed on cDNA obtained from seven individuals and the results were calculated by relative expression and normalized. Bars show the normalized values ± S.E.M.

Figure 3.2: Phylogenetic relationship of EP4 receptors for different species – AsEP4 is Atlantic salmon EP4 receptor.

Figure 3.3: Skin of Atlantic salmon with a sea louse attached. Note loss of epidermis (left part of the picture) while there is more intact epidermis on the right side.
Further to this, in collaboration with WP2 and EWOS, the impact of phytochemicals on the control of salmon louse infection has been studied. Atlantic salmon were fed glucosinolate-enriched diets and had a significantly lower lice infection (a 25% reduction). A manuscript has been submitted.

Yet another study in collaboration between WP2 and WP3 (EWOS/NMBU) was conducted focusing on the expression of immune genes at early time points post lice infection since this has not been studied before as part of WP3 activities. Additional data about the expression of inflammatory and immune cell markers has been generated. The gene expression data has been presented at international conferences (EAFP, Gran Canary and Keystone Symposium, USA). Furthermore, antibodies are currently being tested with the purpose of confirming the gene expression data at protein level using immunohistochemistry. These studies have shown that MHC-II positive cells are present at sea lice attachment sites (skin), and further Mx-positive cells are seen in addition to iNOS-labelled cells. These findings align with the inflammatory and immune profile found by real-time PCR data from the same organ (skin). A manuscript is in preparation and will be submitted after the completion of the remaining work.

In collaboration between NMBU and UiB, a study was carried out focusing on the early time responses of fin tissue to sea lice infection. These responses were contrasted to what was found in skin tissue in the same fish. The most important findings from these studies were that there is higher expression of inflammatory responses in fins compared to the skin, there is significant induction of signatures of classically activated MØ in fins (iNOS, IFN-gamma and CXCL10), and the only gene that was expressed at higher level in the skin compared to the fin was IL-8. The differences are exemplified in Figure 3.4.

**Figure 3.4:** Contrasting responses in fin and skin of sea lice infected salmon. Red indicates upregulation, the more intense, the higher. Bluesh colour indicates downregulation.

**Explore sea lice – virus infection interactions; Test impact of sea lice infection on PD virus susceptibility**

In order to assess the interaction between the sea lice and virus infections, an *ex vivo* model is being developed. The aim is to use the peripheral blood mononuclear cells from sea lice infected, theoretically exposed to the immunomodulatory effect of sea lice, and non-infected fish to assess the impact of the lice infection on the susceptibility and ability of immune cells to respond to virus infection. We have developed a protocol for isolation of peripheral blood mononuclear cells using gradient centrifugation and optimized culture conditions. Incubation media are found to be crucial for cell survival and combinations of mammalian sourced protein and fish sourced protein is crucial. Under optimized conditions minimal cell death is observed up to 7 days post culturing.

**Work Package 4: Molecular parasitology – the basis for novel treatment methods**

**Principle Investigator: Rune Male, UiB.**

Detailed information about salmon lice is essential in order to develop novel tools for future lice control. WP4 includes research in three main areas, copepodid biology, reproduction & endo and exocrine systems. The main aim is to provide a comprehensive understanding of the sea lice biology including life processes to reveal novel targets for development of sustainable drugs and vaccines against the salmon louse.

An integrated approach is probably the best strategy to fight the parasite and obtain a sustainable long-term prevention of sea lice. In such a strategy, we may not see one single method for parasite control, but a combination of new treatment tools such as vaccines, host immune stimulants, parasite repellents, new medicines and other strategies designed to attack the parasite with minimum risks for the environment and consumer. It is also a fact that as the salmon louse is a naturally occurring parasite of wild salmonids, we will never see its total eradication, but will have to develop sustainable methods that can work over a long time scale. Development of new sustainable treatment methods against sea lice depends on detailed knowledge and understanding of the organism. This will require continued efforts in basic research even if the last half of the SLRC will include a stronger focus on output of new treatment methods. In 2015 we have followed the work plan for the year and have recruited two PhD candidates and one post doc. The new personnel will strengthen research in iron metabolism, enzymology and regulation of moulting and ligand regulated ion channels.
Host recognition, chemosensory systems; how sea lice smell salmon

Chemosensory; the ability to detect, differentiate, and respond to chemicals in the environment can be found in all living organisms, and probably arose early in evolution together with life itself. This capability has developed in everything from bacteria to mammals, and gives the capacity to diverse functions such as to sense food from toxic substances and to detect a suitable mating partner. Fundamental similarities in the biology of chemoreception are evident among divergent groups of metazoans, but the dominating type of receptors used in different animal groups may vary. Chemoreception in the salmon louse has been studied in collaboration with Elanco and some of the findings are explored in relation to functional genes in WP2 together with EWOS.

Develop experimental tools and resources

A new assay for initial testing of effect of compounds and chemicals on salmon lice has been introduced. The chemicals were selected in collaboration with WP1 and provided by Elanco. The assay involves exposure during first moulting from nauplius I to nauplius II and scoring of effect a few days later as ability to molt to copepodids and morphology of the larva. The assay does not require the use of fish and operates with small volumes and last only one week.

The genomic sequencing of L. salmonis is finished, but still exploring the transcriptome of single tissues are required. The last year included sequencing of antenna and gland tissues.

Gene transcripts knock down with RNAi

RNA interference (RNAi) is an important defense mechanism against virus and transposable elements and a gene regulatory mechanism found in eukaryotic organisms, from plants to humans. This mechanism has been used to design a powerful experimental method to study the effect of knock down of selected mRNA.

The method include that double stranded RNA (dsRNA) is produced in the laboratory and injected into the use of a very thin glass needle into the haemocel of a salmon louse. Inside the louse, the dsRNA is taken up by cells, probably by endocytosis. Inside the cell the dsRNA is recognized and processed, first by cleavage to short (21–24 nucleotides long) by the Dicer enzyme complex. These fragments may be distributed to other cells aided by the protein SID. The short fragments are further processed by RISC to single stranded molecules that can recognize specific mRNA molecules and direct these for degradation.

The salmon louse chemosensory system is characterized by two groups of proteins. Firstly, the Ionotropic Receptors (IRs) that are associated with smell/taste perception in invertebrates. Since salmon lice lack typical chemoreceptors like the olfactory receptors, IRs are considered to have a key role in the process of chemical communication with the environment, like identification of specific host fish. Secondly, the Serpentine type 7 transmembrane G protein-coupled receptors (GPCRs) that are associated with chemoreception are important in development, reproduction and behaviour.

IRs shows two different patterns of expression. The majority are expressed in chemosensory organs (antennae) and classified as IRs Type A. The subset of IRs designated as type B, have the highest expression in the adult stages, and mainly in reproductive organs. Further investigation of Type A IRs, with the main focus on host recognition, allowed selecting candidate receptors specialized for salmon specific odors. Functional assessment of IRs type B using RNAi expression knock down in pre-adult and adult stages revealed interference with mating processes of both sexes.

Myosuppressin receptors belong to the serpentin 7 transmembrane GPCRs and are related to chemoreceptors. Three Myosuppressin receptors (MS-R 1a, 1b and 2) have been identified in the salmon louse. The MS-Rs bind neuropeptides and are mainly located in reproductive system and intestine. It has been shown that knock out of these genes in insects leads to developmental malformations, increased mortality and disability to reproduce in adulthood and they have been suggested as a good candidate for pest control. RNAi knock-down of MS-R1a caused visible phenotype with lack of spermatophore deposition on the female lice. Localization of expression with in situ hybridization revealed MS-R1a transcripts in the brain, the intestine (Figure 4.1a) and the reproductive system in males and females (female ovaries shown Figure 4.1a).

Myosuppressin receptors are highly expressed in the ovaries. Expression of MS-R1a in adult female salmon lice. (a) In situ hybridization with probe against MS-R1a (blue staining) show high levels of mRNA in the common cytoplasm of early oocytic nuclei. Expression is also evident in the intestine. (b) Negative control. Abbreviations: I – intestine, O – ovaries.

Figure 4.1: Myosuppressin receptors are highly expressed in the ovaries. Expression of MS-R1a in adult female salmon lice. (a) In situ hybridization with probe against MS-R1a (blue staining) show high levels of mRNA in the common cytoplasm of early oocytic nuclei. Expression is also evident in the intestine. (b) Negative control. Abbreviations: I – intestine, O – ovaries.
Specific phenotype was observed in the offspring of animals treated with dsRNA against MS-R1b, suggesting developmental malformations of the body wall (Figure 4.2). Additionally, increased mortality has been observed in all developmental stages (nauplius I, nauplius II and copepodids).

**Figure 4.2:** Knock down of myosuppressin receptor give malformation of the offspring. Phenotypic changes observed in the offspring of animals treated with MS-R1b dsRNA.

**Molting and general growth**

Molting is the process where the old exoskeleton is degraded and a new one is synthesized and this process is regarded as an important target for parasite control. The shedding of the old exoskeleton is the final step in the molting. The main component of the exoskeleton in sea lice is chitin, a long chain sugar polymer. A molting salmon louse has to exit (leave) the old exoskeleton to maintain its growth. The chitin of the old exoskeleton has to be degraded, before new is synthesized. Chitinases are the chitin degrading enzymes, where we have reported three variants with high similarity to enzymes in other crustaceans and in insects. The expression patterns differed between the chitinases during development, pointing to different functions. RNAi knock down gave deformed copepodids not able to infect fish. Chitin synthesis is an enzymatic process that is sensitive to inhibitors (Figure 4.3). The detailed process is not well characterized and is the objective for one of the newly recruited PhD candidates. The first experiments have established an assay for testing of chitin synthesis on free living nauplius larva with compounds provided by EWOS Innovation. The assay works well, is relatively fast and does not need infection of fish.

**Figure 4.3:** Diflubenzuron inhibits molting. Nauplius I (larvae) treated with 0.1 mg/L diflubenzuron (TFB) for 3 hours. Upper row; control animals day 3, 5 and 7 after the treatment. Lower row diflubenzuron exposed larva at day 3, 5 and 7 after treatment. The control larvae molt to copepodid stage between day 3 and 5, while none of the exposed larvae molted even after 7 days.

In all arthropods, steroid hormones initiate a multitude of pathways that regulate different aspects of biological processes such as development and reproduction. The effect of some steroid hormones is generally mediated by binding to a nuclear receptor (NR) complex consisting of two transcription factors; the ecdysone receptor (EcR) and the retinoid X receptor homolog ultraspiracle (USP).

In copepodids, we found the *L. salmonis EcR* (*LsEcR*) transcript to be present in the neuronal tissue, nuclei of muscle fibers and the intestine. Furthermore, we explored the function of *LsEcR* during development using RNA interference mediated knock-down and through infection trials. Knock-down of *LsEcR* in copepodids is associated with hypertrophy of several tissues, delayed development and mortality. In addition, combined knock-down of *LsEcR/LsRXR* resulted in molting arrest during early larval stages.

We have identified the steroid hormones in lice in collaboration with WP1, and the genes that code for the necessary enzymes in the steroid hormone synthesis; octopamine receptor, neverland, disembodied and shade. Functional studies of the genes using RNAi in nauplius showed that octopamine receptor and shade are necessary for successful molting from the nauplius II to the copepodid stage.
Reproduction; germ cell differentiation and maturation

Basic information about gamete production and maturation are essential in order to understand how reproduction can be utilized in future parasite control. Lipids are important as a major source of energy for sea lice, make up membranes and are important signaling molecules. Embryos inside egg strings, the free swimming nauplii and copepodids before host attachment are all totally dependent on maternally deposited lipids. The lipid content of nauplii larva is mainly found in the yolk sack and is rapidly reduced to one or two oil droplets in copepodids (Figure 4.4). The maternal lipid storage is built up in vitellogenic oocytes as many small oil droplets, while the ovaries only have relatively small lipid deposits (Figure 4.5).

Figure 4.4: Lipids are stored in droplets in sea lice larva. Oil Red O staining of lipid droplets in a copepodid. To demonstrate the presence of lipids in tissues of sea lice, the standard lipid stain Oil Red O has been used to detect neutral lipids. Most of the ORO staining was confined to the unfertilized eggs and in the yolk mass of copepodids.

Figure 4.5: Large amounts of lipids are stored in vitellogenic eggs. Adult female and dissected ovary from the head region and unfertilized egg string from gonadal segment were treated with standard lipid stain Oil Red O to detect neutral lipids. Most of the ORO staining was confined to the unfertilized eggs in the yolk mass of copepodids.

A large lipid transfer protein superfamily (LLTPs) has been identified and characterized in both vertebrates and invertebrates. This protein family includes vitellogenin, vertebrate apolipoprotein B (apoB), insect apolipoprotein-II/I (lipophorin) and microsomal triglyceride transfer protein (MTP) and plays an important role in animal reproduction and energy metabolism. Developing oocytes accumulate large amounts of lipids in addition to proteins necessary for development of the future embryos. Vitellogenin (Vtg) is the main yolk protein precursor present supplying nutrients including lipids to developing oocytes. In addition to Vtg, insect’s lipophorin (LP) also works as a general lipid transporter to various organs especially to growing oocytes. MTP plays an important role in the formation of nascent lipoprotein particles in vertebrates and apolipoprotein-II/I in insects. To understand their role for lipid delivery to growing oocytes in sea lice, MTP and LP were characterized. Expression studies showed high levels of LP in the sub-cuticular tissue and intestine, whereas MTP is highly expressed in all tissues except intestine.

MTP knock down in salmon lice using RNAi (93% downregulation) resulted in the absence/abnormal egg strings and significant mortality of copepodids was observed after hatching that could indicate energy deprivation.

The function of LP was studied using gene knockdown. Not all, but most of the RNAi treated females had short egg strings as compared to control animals. Quantitative real time PCR was performed to measure the down regulation of Lp in RNAi treated and control animals. Lp down-regulation was found around 65%.

Immunomodulation, exocrine activity

Detailed knowledge about host parasite interaction is important in order to find novel ways for parasite control. In this respect basic information about anatomical structures and the function of these are creating a fundament for future innovation. Exocrine glands of parasitic copepods are believed to be important in maintaining the tegument (the outer covering of the lice), digestion of food and in the host-parasitic interaction by secretion of substances that modulate the immune response of the host and limit clotting of the blood. It is important to know more about these glands in salmon lice, as they may offer understanding of host immune suppression and possibly vaccine candidates. Three types of tegumental (empties on integument surface) (teg 1-3) glands and a labial gland (around the orifice of the mouth) type are identified. Teg 1 and teg 2 glands are found from the nauplius I stage, and are suggested to be important in maintaining the tegument. Teg 1 glands are the most numerous types, and express three marker genes that indicate further division into subtypes. One marker gene has also been identified for teg 2 glands. The location of the teg 2 glands and their secretory pores suggest a function in lubrication of movable joints. Thus, it is suggested that teg 1 glands are important in maintaining the tegument in general, while teg 2 glands are important in exposed areas in need of a higher maintenance. The labial glands (Figure 4.6A,B) have secretory ducts that extend into the lower lip (labium) of the salmon lice and empty into the oral cavity. The labial gland was first identified at the planktonic copepod stage, making the copepod ready to start feed-
ing once it settle on its host. The labial glands were found to have reservoirs that allows for a controlled release of glandular products during feeding. The secretory reservoirs are seen filled with proteins visible as blue aggregates in Figure 4.6B. The teg 3 glands (Figure 4.6A, D) were not identified before the lice enter the preadult stage. At this point in development the lice reaches a virulent phase, capable of inflicting substantial damage to the host. Moreover, teg 3 glands have secretory ducts ending at the host-parasitic contact area (Figure 4.6A,C). Hence, the teg 3 glands secretions may be an important virulence factor for the lice. Further analysis on the glandular products of teg 3 and the labial gland has been initiated, as these gland types are likely to be especially important in the host-parasite interaction.

Figure 4.6: Characterization of exocrine glands. A) An adult female lice, ventral side; the side facing the fish. Circles indicate the location of teg 3 glands. B) Morphology of the labial glands that have secretory ducts within the oral cavity of the lice. The gland reservoirs are seen with light blue aggregates. C) The pore opening of a teg 3 gland in the marginal membrane. This membrane is meeting the fish skin, and the teg 3 glands secretion is thereby secreted directly on the fish. D) General morphology of a teg 3 gland.

In Atlantic salmon the immune response is not sufficient to expel the parasite during Lepeophtheirus salmonis infestation. It is therefore expected that the salmon louse excretes immune modulating substances. Some parasites are found to modulate the immune defense of their hosts by the secretion of prostaglandins. Interestingly, prostaglandin E2 has been found in excretions of the salmon louse. The prostaglandin E synthase 2 (LsPGES2) was sequenced, compared to prostaglandin E synthases from other species and its expression was monitored. Comparison showed high similarity to enzymes from other crustacean species while similarity to enzymes from other blood feeding organisms was not found. LsPGES2 is relatively stably expressed in all developmental stages, but highest in free living stages and in adult female lice. In situ hybridization detected expression in nauplius abdomen and cephalothorax, in copepods in tissue surrounding muscles, while in adult female lice expression was found in the ovary and the oviduct as well as in immature oogonia. In collaboration with researchers in WP3, LsPGES2 was knocked-down by RNAi in pre-adult female lice, which were kept on the host until they were egg producing adult lice. Prevalence of lice on fish and their reproductive success was compared between knock-down and control lice. No difference was found. Knock-down was also implemented in nauplia and used to infest fish. No difference in abundance and development of lice between knock-down group and control could be found.

Novel treatment targets – vaccine candidates
Based on our RNAi screening and additional data on expression patterns several candidate genes have been identified that will be further evaluated as vaccine target through clinical trials. In close collaboration with ELANCO several vaccine candidates are currently being expressed in vitro in order to produce antigens for test vaccines. The first vaccination studies will take place in collaboration with University of Prince Edward Island and the clinical studies will be initiated early in 2016.

New medicines
In close collaboration with Elanco, several chemicals known as juvenile hormone analogues and insect growth regulators have been tested as ligand for ecdysone receptor (EcR) and for ability to modify/block molting in larva. None of the chemicals proved to act as direct ligands for EcR, but two of them disturbed molting at low concentration indicating alternative pathways for regulation of the process.
Work Package 5: LiceBase

Principle Investigator: Inge Jonassen, UiB

An important part of our infrastructure/resources for innovation towards novel tools for sea lice control is based on utilizing the salmon louse (and other relevant species) genome and LiceBase is created for this purpose. The focus of WP 5 is to provide excellent bioinformatics resources for the analysis of the sea lice genome and related genomic data to the other work-packages, and in the continuation to the international research community. LiceBase is a web-database providing users with access to the most recent genome assembly of the *L. salmonis* genome and the corresponding Ensembl annotation. During the last years, extensive additional functional annotation of proteins, genes, and other genomic features has been integrated. Since its upstart in 2014 LiceBase has become the core platform and an invaluable tool to the SLRC for integrating results from high-throughput and complex reverse genomics experiments (e.g. RNAi), further linking those with external resources.

Bioinformatics is at the heart of modern biology

In order to understand the biology of a parasite (or any other organism) and its interaction with the host, the genome is invaluable and serves as a source of information on genes and systems present in the organism as well as those that are missing. Molecular level data generated to study gene expression, genomic variants linked to different phenotypes, etc can be linked with the genome and re-used in other contexts later – and be valuable both for answering sea louse specific biological questions but may also be useful for more general comparative studies. In this work package we build LiceBase to be both a resource for the centre but also aiming to build a resource for the wider community. A public version of licebase will be launched once the genome becomes public, likely in the spring of 2016.

Advanced requirements

Following an iterative development cycle, with strong focus on user suggestions and feedback obtained mostly from training workshops held in 2014, the LiceBase.org portal was mostly perceived as sufficiently feature rich and complete. For this reason, while we continued working with updates that improved the user experience, usability and user communication, we decided that more input for new ground breaking functionality was to be generated within WP5 itself or be inspired by other life-science portals. Further propositions were adopted from the Mid-term evaluation report.

As a result, three areas of high potential for increasing the overall benefit of the site have been identified:

- advanced data-mining capabilities for high-throughput experiments and annotation, resulting in the specification of the a new project: The Atlas of Gene-Expression for salmon lice
- advanced features for creating sets of genes-of-interest (GOIs) and bulk-export of sequence data, resulting in the specification of the Gene-basket
- advanced features for data-storage and analysis that are accessible users without experience in command-line tools, programming, or statistics; hence we intensified the integration with ELIXIR.NO and specified a RNA-sequencing pipeline for *L. salmonis*, that would allow non-computational staff to analyze their own data efficiently.

Ontologies for developmental stages and gross anatomy

An ontology for annotating all developmental stages of sea lice was finalized in 2012. The next step according to the initial plan was creating an ontology for the conceptual representation of anatomical structure, organs and body parts. However, we chose to down-prioritize this task, due to overall low interest from the center. Possibly, we overstated both the demand for such an ontology and the available knowledge about the anatomy of sea lice in the initial project plan. In the light of other more urgent tasks we suspended the anatomy ontology. However, the Sea Lice Ontology Working Group (SLOW) which has been versatile for implementing the developmental ontology in 2012 might be picked up in the future if we see the need.

Updates and new features in the LiceBase.org portal

The LiceBase.org portal has been available to the center since 2014, and since then has become a core tool to the center for genome research and high-throughput data of salmon lice. The lice genome and its most recent annotation are maintained in LiceBase and serve as a reference point to other SLRC resources, including lice microarrays and NGS data such as DNA and RNA sequencing, RNA-interference experiments. LiceBase.org features a community-wide genome annotation process, providing tools for updating gene annotations, uploading new sequences and reporting potential errors in the genome assembly and annotation.

In 2015, analysis pipelines for center members were set up, first focusing on analysis of RNA-seq data. The pipelines are available to center members via the NeLS (Norwegian e-infrastructure for Life Science) web portal and have been set up in collaboration with the ELIXIR.NO project (Figure 5.1).

In order to facility tight integration with resources from ELIXIR.NO, LiceBase.org for federated authentication and authorization has been enabled. LiceBase.org was registered as a service provider with Feide (feide.no), the identity provider for all Norwegian educational institutions. By using Feide, we enable Single-sign-on and Single-log-out, using the same credentials for all Feide-enabled services, and reduce the administrative overhead for user registration, password recovery etc. We have also implemented a solution for interested users without access to a Feide account (e.g. for temporary use in courses, employees of industrial partners, or international collaboration partners).

Further newly implemented features include the ‘Gene basket’ which is inspired by online-shopping solutions, a similar implementation is found in the Uniprot database and the legume information system (legumeinfo.org). A gene basket allows users to define custom collections of GOIs (genes of interest), to export sequences in various formats. The gene basket for the LiceBase.org portal was implemented as student programming project during a summer internship and offers multiple sequence alignments and phylogenetic analysis of the sequences in the basket using web-services. The basket will be made available in the next release of LiceBase.org in 2016.
We have also initiated the development of the Atlas of Gene Expression in Sea Lice an integrated data-mining solution which complex queries across functional annotation and gene expression data. We are implementing this data-mining tool with the intention to facilitate the discovery of vaccine candidates and potential drug targets. As an example, a query for vaccine targets to the system could be phrased as “Retrieve all protein sequences that are highly expressed in the intestine AND have at least two trans-membrane domains AND at least one non-cytoplasmic domain”. The new functionality will be topic for workshops with academic and industrial partners within the SLRC – which will also give us input for further development of this functionality. The development of the Atlas is part of an ongoing PhD project started in 2015, and the first version is expected to be available in the portal for 2016.

The major inhibitory neurotransmitter in the mammalian central nervous system is gamma-aminobutyric acid (GABA), the pathway is also almost fully present in the salmon louse. Proteins for which orthologs in the louse have been detected are highlighted in red.

Figure 5.1: Reconstruction of the GABAergic synapse pathway in the salmon louse.

Analysis

The main focus for 2015 was to develop data-mining and interactive genome annotation tools to foster the community annotation process, and the support of reverse genomics techniques. As a first step, we have manually assigned function description and gene names to over 100 L. salmonis genes during 2015. By the end of the year, there were over 200 annotated RNAi experiments stored within the database. WP5 has contributed to the quality assurance process for these annotations by checking the data for completeness. We have specifically worked with ensuring each experiment has both an annotated target and a correctly annotated phenotype observation.

Members of WP5 have closely collaborated with other work packages on the analysis of data. We helped with importing validated cDNA (RACE-PCR) sequences and performed and imported resulting alignments which lead to new validated gene models. We were also involved in the analysis of the membrane topology of ionotropic receptors (WP4), and new RNA-seq data (WP2, 4), and the preparation of an approach to sequence all microRNAs expressed in the salmon louse. The prediction of Iron Responsive Elements in the genome of L. salmonis led to the publication of one paper.

The SLRC has closely reviewed the recommendations given in the Mid-Term Evaluation report, to attribute additional focus to the discovery of vaccine or drug targets. Development of vaccine targets against ectoparasites, not to speak of their automatic prediction from the genome sequence, is vastly undiscovered terrain, with only a single vaccine (against the cattle tick Boophilus microplus) ever commercially released. We have, however, worked to identify a set of criteria for potential computational prediction of anti sea lice vaccine candidates that could be validated in an iterative process, and have identified one potential candidate. Our data mining solution (Atlas) will become a key tool to identify more candidates via a bioinformatics pipeline.

Figure 5.2: Overview of the implemented RNA-seq workflow specific to sea lice data in the NeLS Galaxy hosted in Bergen. The galaxy workflow allows efficient ‘do-it-yourself’ analysis of RNA-seq transcriptomics data. The raw reads are first aligned against the LSALAT2s reference assembly using the fast and sensitive software RNA STAR, counts per Ensembl transcript are then calculated from the resulting alignments using featureCounts.
Work package 6: LiceLab

Principal investigators Lars Are Hamre, UIB and Sussie Dalvin, IMR

The experimental facilities are the fundament of a Centre like the SLRC, where LiceLab contributes by providing state of the art infrastructure and expertise to study sea lice and host parasite interaction. The Lice lab facilities are situated at the High Technology Centre in Bergen (UiB), at Institute of Marine Research (IMR) and at Ewos Innovation in Dirdal. The facilities have a unique capacity to study sea louse biology, to perform large scale efficacy assays and RNAi experiments as well as capacity to cultivate material for research and to maintain lice strains with specific properties. This is of vital importance to:

• obtain detailed knowledge of sea louse biology in order to identify treatment targets
• efficiently discover, evaluate and develop new medicines
• effectively screen vaccine candidate targets and evaluate test vaccines
• identify drug resistance mechanisms and establish tools to determine optimal use of medicines
• evaluate effects of non-medicinal methods

The activities in WP6 are divided in four main areas:

• Sea lice lab facilities
• Production of sea lice strains and experiments
• RNAi screening
• Production of lice and experiments at EWOS facilities

Sea lice lab facilities

Wet labs at UiB and at IMR have been upgraded in 2015. Among the 114 single fish tanks at UiB, the number of tanks fitted with separate water supply/outlet has been increased to a total of 90. This enables direct infection on each individual fish using copepodids, allowing for more efficient and versatile use of the tanks. At IMR the single fish tanks have been upgraded to the latest design and more incubators have been built.

Production of sea lice strains and experiments

A total of nine sea louse strains were maintained in 2015, hereunder three sensitive strains, one inbred strain and strains resistant to various medicines and/or multiresistant strains. Material for in vitro experiments and RNA and DNA purification was produced and sampled for academic partners in Bergen and Oslo, serving about 35 researchers/phd’s/master students with material for ongoing research.

*Caligus elongatus*, in Norwegian termed ‘skottelus’, is a generalist fish louse with a wide host record and with a biology that in some areas are very different from the salmon louse. This fish louse has received some attention recently due to a higher occurrence in salmon farms in parts of Norway in 2015. In order to learn about its biology and to gain knowledge on how to breed and design experiments and efficacy studies with this sea louse, lice lab has for some time maintained test cultures of *C. elongatus*. Through this work we have established a knowledge base for cultivating specific strains and how to do wet lab experiments with this species of sea lice. Lice material from the test cultures was made available to NMBU to determine baseline levels of *C. elongatus* sensitivity to a range of commercial delousing chemicals.

An in-vivo drug assay for orally administered compounds was successfully developed and three compounds were tested in collaboration with Elanco. The assay design allow for high throughput in vivo screening of new medicinal compounds, evaluating both therapeutic and long term effects against sea lice using a minimum of time and experimental fish. This will hopefully reduce the overall development time of novel salmon louse medicines. Full scale screening of promising compounds commences early 2016.

F2 hybridisation experiment with SLICE: in order to attempt to identify the mutation(s) causing resistance to slice, two F2 hybrid crossing experiment was initiated. The overall aim was to produce F2 hybrid lice which could thereafter be used to identify the genomic regions linked with this resistance.

**Figure 6.1:** Adult male and female sea lice.
Lice material and lab capacity was also provided to other research groups at the Institute of Biology, UIB. The SLRC hatchery and incubators was made available for the master project “Intensive aquaculture: Life history responses in energy allocation towards offspring in salmon lice (Lepeophtheirus salmonis)”. The SLRC also provided copepodids for the master project “Salmon (Salmo salar) infected by salmon lice (Lepeophtheirus salmonis) become more susceptible to new infections”.

How to vaccinate a salmon against salmon lice
The idea behind vaccination is the same in fish as in humans or in other animals. The disease causing agent is introduced into the fish in a harmless form and the immune system in the host will in this way learn to recognize the disease. Once the immune system has thus been trained, it will quickly be able to fight the real disease when exposed later on and not get sick. To study the effect of salmon lice vaccines, we typically inject salmon with small pieces of salmon lice proteins. Some fish are injected with true vaccines whereas other groups are injected with placebo vaccines acting as controls. After injection the fish is left for about eight weeks to develop an immune reaction. Then we expose the fish to salmon lice copepodids and then after another three to eight weeks, we can assess the number of surviving lice. If the vaccine works, we should find significantly less lice on the vaccinated fish compared to the control fish.

RNAi screening
Silencing or knock-down of a gene provide crucial information about biology and serve as a tool to evaluate any target as a target for treatment. Three types of RNAi screens were carried out in the wet lab; I) RNAi in preadult II females and II) RNAi in nauplius, III) RNAi in embryos (egg strings). RNAi in preadults was performed by injection of preadult II females, after which the lice were placed back on fish. When the experiments were terminated, phenotypes were scored on a morphological basis inspecting whole animals and in some cases sections of these. Phenotypes included lack of development, termination of reproduction, decreased digestion, behavioral changes and mortality. Nauplius RNAi was performed by bath treatment of nauplius I animals followed by incubation in seawater. At termination, animals were harvested as free-living copepodids. In some cases copepodids were transferred to fish to monitor further development. Experiments with RNAi in embryos were initiated, further evaluation of this method proceeds in 2016.

Evaluation of RNAi screens from 2015 is on-going and results on specific genes will be reported from WP4. An overview of all experiment and results obtained is summarized in the table below.

### Table 1: RNAi screens in 2015

<table>
<thead>
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<th>Year</th>
<th>Method</th>
<th>Total RNAi screen</th>
<th>Total gene targets</th>
</tr>
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<tbody>
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</tr>
<tr>
<td>2014</td>
<td>Preadult</td>
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</tr>
<tr>
<td>2013</td>
<td>Naup</td>
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<td>29</td>
</tr>
<tr>
<td>2012</td>
<td>Preadult</td>
<td>76</td>
<td>62</td>
</tr>
</tbody>
</table>

### Figure 6.2: RNA interference performed in nauplius larvae to investigate the molting process. The picture shows a nauplius larva that has been treated to inhibit molting and the larva is arrested in the nauplius stage unable to molt into an infective copepodid.

Production of lice and experiments at EWOS facilities
Lice production was increased during 2015 and populations made available for other sea lice collaborators at NMBU, as well as for WP2 and WP3. Lab facilities were developed for ex vivo methods (as described in WP2 annual report), requiring additional resources for fish maintenance and culture methods. The building of the new sea lice lab in Chile continued throughout 2015 and will be operational during the second quarter of 2016.
ASSOCIATED PROJECTS

One of the success criteria for an SFI is the ability to obtain additional funding and collaboration with other organisations. During the first part of the Centre, additional research projects have been applied for and the rate of success has been good. The table below shows the variety in topics, funding and collaborating partners, and the most important ones for 2015 is presented briefly:

‘Sprøing av lakselus’: copepodids of a fully sensitive strain (LsAlta) were produced (n=400 000) and used to infect fish farms in Bjomafjorden and Hjeltefjorden to study the subsequent dispersion of their offspring to neighbouring farms. The infection with copepodids in the fish farms showed a variable success, in one instance very successful and in one instance probably hampered by a high density of small jellyfish. Offspring from these lice were later retrieved from farms at various distances away from the farms originally infected.

Genetic variation of salmon to sea lice infection: approximately 160 000 LsAlta copepodids (strain fully sensitive to all delousing chemicals) were produced in a collaboration between SLRC and IMR and used to infect a single test cage at a Marine Harvest farm. The aim of the study was to investigate the level of genetic variation in the Mowi salmon strain to sea lice infection.

‘Merdvariasjon’: Studies done at the Sea Lice Research Centre (SLRC) have enabled the development of new diagnostic methods for monitoring resistance traits towards chemothapeutants in the salmon louse. These diagnostic methods have been developed to be a part of PatoGen Analyse’s services to the aquaculture industry. The primary goal of the present project is to provide new data on the occurrence of resistance traits on farms, and in pens within farms, and to elucidate relationships between treatment effects and the occurrence of resistance traits towards given chemotherapeutants. The project will also contribute with data that will enlighten questions regarding treatment-induced selection of resistance traits in farm-populations of salmon lice. So far, the project has revealed that there is a predictable change in pre- and post-treatment occurrence of genetic markers associated with organophosphate resistance in farm populations of salmon lice. This also seems to be the case for pre- and post-treatment occurrence of markers associated with pyrethroid resistance, although this has been more difficult to demonstrate since pre-treatment occurrence of resistance markers generally are high in Norwegian farm populations of lice. For hydrogen peroxide, relationships between treatments and the occurrence of genetic markers have been less predictable. Pen level variation in resistance traits and treatment effects will be the focus of the remaining part of the project. Partners in the project are: Marine Harvest ASA, Lerøy Seafood Group ASA, Grieg Seafood ASA, Patogen Analyse AS andUiB.

Genome-based improvement of salmon sea lice resistance: The main objective for the project is to implement genome-based selection tools to efficiently improve sea lice resistance in Atlantic salmon, and to establish a knowledge base for further studies of host-parasite interaction at the genome level. For that purpose genetically resistant and susceptible salmon was constructed for use in controlled common garden experiments targeting natural resistance against L. salmonis. Functional genomics studies of salmon-louse interaction will identify genes and pathways involved in resistance to sea lice in the salmon, as well as identify differential gene expression responses in the sea lice when exposed to a susceptible versus a resistant host and by that contribute to a better understanding of the biology underlying the host-pathogen interaction. The project is a part of Havbruksprogrammet/RCN and will be finalized by the end of 2016. Partners in the project are NMBU (2), Aqua Gen and UiB.

The table below shows how the SLRC partners have received funding for associated projects.

<table>
<thead>
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<th>Funder</th>
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<tr>
<td>FHF</td>
<td>2 333 345</td>
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<tr>
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<td>Public organisations</td>
<td>222 367</td>
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<tr>
<td>Total</td>
<td>7 491 446</td>
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</table>
International collaboration

During 2015, international cooperation has been developed directly from the centre activities but also based on new and existing individual networks.

The SLRC has been building up the international collaboration along two lines. International collaborating projects like projects from EC is one way of building up international network for collaboration. Another way is developing and expanding existing networks through new bilateral contacts. Several proposals have been submitted in 2105 to obtain both international and national funding, and collaboration with organisations outside Norway has been in focus.

The SLRC and the individual partners are leading scientists within their field and are attractive for international collaboration. A major plan for future years is to include the younger scientists in the existing networks, and to encourage them to expand and to create their own networks. The industrial partners in the SLRC are international companies and collaboration is an integrated part of their business. During the year, SLRC PhD-students and researchers have taken part in some of these activities related to the work in the centre. Highlights from the international activities in 2015:

Cross Atlantic Sea Lice, CASL – a project in the Intpart program

In 2015 researchers at the SLRC obtained funding for a 3-year project from the NRC and SIU to enhance collaboration between the SLRC and researchers at University of Prince Edward (UPEI) Island and University of Vancouver (Canada). The Norwegian Seafood Innovation Cluster is also a partner in CASL. Funding will be used both to establish formal collaboration between the institutions (UIB, Atlantic Veterinary College (UPEI) and University of Vancouver) and for individual mobility of students, post doc and researchers as well as communal workshops. The project starts in 2016 and is planned to last for 3 years.

Visitors to and from the SLRC

The SLRC is an attractive centre to visit, both for educational, scientific and industrial purposes. In 2015 master students and PhD students from

• University of Groningen, Netherlands
• Polytech Université de Clermont Ferrant, France
• University of Concepción, Chile
• King Mongkut’s University of Technology Thonburi, Thailand

have been visiting the academic partners through various mobility programs like ERASMUS and IAESTE or bilateral agreement. The SLRC would like to share the knowledge established in the centre and welcome representatives from universities, public organisations and companies to visit the SLRC and LiceLab. During 2015 The Thai Union Group and King Oscar AS, The Indonesian Ambassador and The Canadian Ambassador have visited the LiceLab. A goal for presenting the facilities is to establish new contacts and lay the foundation future collaboration.

The PI of WP3 spent a sabbatical at Stanford University, School of Medicine, Palo Alto, California, USA, from August ’14 to August ’15. Focus has been on anti-viral targets of host cells. This has relevance for the lice-host interactions, particularly modulation of susceptibility to virus infection (in salmon) and cellular targets of infected fish of either pathogen potentially converge.

Chilean collaboration

There is a close collaboration between partners in the SLRC and both Universities and farming industry in Chile. NMBU scientists have visited the University of Concepcion in Chile to process a range of samples (Atlantic salmon and L. salmonis) using RNA-seq techniques. Further collaboration will be developed with this group to progress the use of RNA-seq. Further to this the PhD candidate contributed to launch of anti-attachment sea lice product in Chile. In addition, representatives from UiB have presented the SLRC for the Chilean ambassador in Norway.

The CORNYCT funded project in Chile, assessing lice responses to different host species was completed during 2015 and the final report submitted. This highlighted the key defensive mechanisms of the resistance pacific species. The activity will be conducted with valuable support from Laura Braden at the University of Victoria and Atlantic Veterinary College, Canada.

SLRC personnel from NMBU and EWOS Innovation AS attended the launch of the new EWOS anti-lice feed in Puerto Varas in Chile, where findings from WP2 and WP3 were presented to scientists, customers and other representatives of the Chilean salmon industry. After the launch, the SLRC personnel paid a 3-week long visit to the lab of prof Cristian Gallardo at the University of Concepcion to process a range of samples (Atlantic salmon and L. salmonis) using the RNA-seq technique. To progress the use of RNA-seq, further collaboration will be developed with this group to progress the use of RNA-seq data analysis in Oslo.

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collaboration with this group took place in autumn 2015 where staffs from Gallardo’s lab attended the PhD course on salmon lice biology organized by the SLRC in Bergen followed by a week of RNA-seq data analysis in Oslo.

The main international activity for the SLRC is in combination with dissemination of results in the centre. Throughout the year, researchers and industrial partners have presented the research in the centre at a broad range of international meeting and conferences. This activity is a valuable base for new international networks and collaboration.

RECRUITMENT

The PhD student and postdocs employed at the start of the SLRC in 2011 and 2012 are now close to finishing their work. As half of the SLRC centre period passed in September 2015, and the centre will get funding for the last period, the recruitment of “2nd generation” PhD students and Postdoc has started. Some have been employed from October 2015, but the major part of new SLRC-people will start their work in January 2016. Some of the new PhDs participated at the International PhD Course in Sea Lice Biology arranged in October by the SLRC and the Molecular and Computational Biology Research School at UiB.

Sussie Dalvin has from August 2015 and three years been engaged as Associated Professor (20%) at the Department of Biology (BIO), UiB. Sussie Dalvin has her main position at IMR as researcher, and is one of the WP-leaders in SLRC. The SLRC has together with BIO applied and received funding for the position from the UiB program in gender equality means. The SLRC will be a co-funder of the position, and is regarded as a valuable tool for recruitment of females above PhD and Postdoc level in the SLRC. Dalvin is also enrolled in “Balanse – Bergen”, a project in gender balance in senior positions and research management in Bergen. She is transferring the knowledge on how to plan and build your career for females in the SLRC through meetings with PhDs and Postdocs.

Alltogether four master students have finalized the master programme at UiB and NMBU in 2015, and five new students have been recruited for 2015/2016.

There has been minor changes in the SRLC personnel during the year, and by the end of 2015 the balance between the genders is 53.3% females and 46.7% men, which is a marginal increase in females compared to 2014.

The overall gender aspect in the SLRC is in accordance with the strategy outlined in the project description and satisfies the requirements for female-male ratio. As not all the key personnel are working full time for the SLRC, the total number of man-years for 2015 is calculated to 23.25 – including personnel at the user partners.

DISSEMINATION

One of the success criteria for an SFI is to conduct high quality research. Publishing in well recognised journals and participation at conferences and meetings is the best way to show the excellent science in the SLRC. In 2015 researchers from the SLRC have published 15 journal papers, and given 31 presentations external scientific presentations. Principal researchers in the SLRC have been invited as key note speakers to international conferences, both in Europe and Chile.

In addition the SLRC had 42 media coverages, where a presentation of the centre and interview with the centre director Frank Nilsen in BBC News reached a wide audience.

Companies and organisations related to the salmon farming industry are among the main target for dissemination of activities and results taking place in the SLRC, and 2 open seminars have been arranged in 2015. The first was and international seminar 9 June in Bergen where people from industry and academia participated. The second was arranged as a breakfast to lunch meeting in Trondheim 20 August – in conjunction with the Aqua Nor 2015 Conference. The focus was more dedicated to challenges for the industry.

A large and diverse audience is generally interested in sea lice through interests in salmon farming, wild salmon or environmental issues. In 2015 both researchers and students in the SLRC have presented the centre and various arenas, such as:
Christiekonferansen 2015

The Christie Conference aims to be the most important meeting point between academia and the society in the western Norway. Approximately 350 participants attend the conference in Grieghallen, Bergen at the end of April. The North Sea was the overall topic for the conference, both in a marine, climate and political perspective. The SLRC was invited together with 5 other excellent research groups to present the centre and the scientific work developing from it. The conference had a wide diversity of participants and many had their first experience with live salmon lice. In the beginning of 2015 the centre made a film about the scientific areas in the SLRC in combination with results and information in a societal context. The film was shown for the first time at the Christie Conference.

Forskningsdagene 2015

“Food” was the main topic for the National Science Week 2015, and the SLRC had together with The Institute of Marine Research a 2-day stand at Bergen Science Fair in September. For 2 days, around 6000 Schoolchildren and adults visited the Science Fair. The Science Fair is an excellent opportunity to communicate activities of the SLRC to the society and to provide information about the parasite and the various problems salmon louse causes. Microscopes, films, and live lice were used by the SLRC staff to give visitors (both adults and children) a unique experience.

The SLRC has arranged two open lectures in 2015, both in connection to meetings with international collaborators to the centre. Both lectures have been given at UiB during fall 2015: Professor Simon Jones visited the SLRC and Bergen in October to give a lecture on “Sea Lice ecology from a pacific perspective: ecology and host interaction”. Jones is the lead scientist in the finfish parasitology program at DFO’s Pacific Biological Station in British Columbia, Canada.

Dr. Paul Kersey, team leader at The European Bioinformatics Institute, EMBL-EBI, visited Bergen in November and gave a lecture on the landscape of known genomic data, and associated challenges: “Future Directions in Genomics Infrastructure”.

Summary of SLRC dissemination activities:

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### ATTACHMENT TO THE REPORT

#### Personnel Sea Lice Research Centre 2015

**Key Researchers**

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<tr>
<td>Frank Nilsen</td>
<td>UiB</td>
<td>WP1, WP4</td>
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<tr>
<td>Susie Dalvin</td>
<td>IMR</td>
<td>WP4, WP6</td>
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<tr>
<td>Rune Male</td>
<td>UiB</td>
<td>WP4</td>
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<td>Tor Ivar Hanberg</td>
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<td>Vegar Jonassen</td>
<td>UiB</td>
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<td>Sindre Grosnom</td>
<td>UiB</td>
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<td>Christiane Eichner</td>
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<td>Michael Dondrup</td>
<td>UiB</td>
<td>WP4/WP6</td>
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<td>Kevin Glover (Professor II)</td>
<td>UiB</td>
<td>WP4/WP6</td>
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<tr>
<td>Peder Jansen (Professor II)</td>
<td>UiB</td>
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#### POSTDOCTORAL RESEARCHERS WITH FINANCIAL SUPPORT FROM THE CENTRE BUDGET

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<td>Arya Cemal*</td>
<td>Sudanese</td>
<td>01.04.12–08.03.17</td>
<td>M</td>
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<td>Stanko Skupor*</td>
<td>Croatia</td>
<td>01.06.12–31.05.17</td>
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<td>Mart Bakke*</td>
<td>Norwegian</td>
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<tr>
<td>Aina-Cathrine Øvergård</td>
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<tr>
<td>Melanie Andrews</td>
<td>Norwegian</td>
<td>01.08.14–31.07.18</td>
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* Researchers working at Post doc. level

#### POSTDOCTORAL RESEARCHERS WORKING ON PROJECTS IN SLRC WITH FINANCIAL SUPPORT FROM OTHER SOURCES

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<tr>
<td>Kari D. Helgesen</td>
<td>Norwegian</td>
<td>01.08.15–31.06.18</td>
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<td>Celia Augusti-Riidsa</td>
<td>Spanish</td>
<td>01.07.14–30.06.16</td>
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<td>Christopher Haves</td>
<td>Chilean</td>
<td>09.06.12–01.12.15</td>
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<td>Christerine Tröse</td>
<td>German</td>
<td>01.09.11–01.05.15</td>
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<td>Jon Anders Stavang</td>
<td>Norwegian</td>
<td>01.01.12–25.03.15</td>
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#### PHD STUDENTS WITH FINANCIAL SUPPORT FROM THE CENTRE BUDGET

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<tr>
<td>Liv Sandlund</td>
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<td>Mohammad T. Kahn</td>
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<td>28.08.12–25.02.17</td>
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<td>Stan Marsh Asem</td>
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<td>Helle Holm</td>
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<td>Jowai Haraszczuk</td>
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<td>Zhaoyan Zhou</td>
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<td>Ina Irene Hegglund</td>
<td>Norwegian</td>
<td>26.10.15–25.10.19</td>
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<td>Huld Hardardottø</td>
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<td>26.10.15–25.10.19</td>
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<tr>
<td>Helene Baranen</td>
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#### MASTER DEGREES

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<tbody>
<tr>
<td>Andreas Berge</td>
<td>Norwegian</td>
<td>2014/15</td>
<td>M</td>
<td>WP4/WP6</td>
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<tr>
<td>Sergio Cardoso da Rocha</td>
<td>Portuguese</td>
<td>2014/15</td>
<td>M</td>
<td>WP4</td>
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<td>Henriette Wangen</td>
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<td>Sukana Kar</td>
<td>Nepali</td>
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<td>Joakim Brunet</td>
<td>Norwegian</td>
<td>2015/16</td>
<td>M</td>
<td>WP4, Mamata</td>
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<tr>
<td>Nomara Iqbal</td>
<td>Pakistani</td>
<td>2015/16</td>
<td>F</td>
<td>WP4</td>
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<tr>
<td>Syed Abideen Noor</td>
<td>Pakistani</td>
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<td>Joao Paulo Barbosa</td>
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<tr>
<td>Nuar Leikvoll</td>
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<td>2015/16</td>
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<td>WP4</td>
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TECHNICIANS WITH FINANCIAL SUPPORT FROM THE CENTRE BUDGET

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<tr>
<td>Lars Are Hamre</td>
<td>Norwegian</td>
<td>01.09.11–</td>
<td>M</td>
<td>WP6</td>
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<td>Bjørnar Skjold</td>
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<td>01.07.15–</td>
<td>M</td>
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<tr>
<td>Per Gunnar Espedal</td>
<td>Norwegian</td>
<td>01.04.13–31.08.16</td>
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<td>WP6</td>
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<tr>
<td>Hendi Kongshaug</td>
<td>Norwegian</td>
<td>15.06.2012</td>
<td>F</td>
<td>WP4</td>
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<tr>
<td>Lourdes Tan (10%)</td>
<td>Norwegian</td>
<td>01.01.12–</td>
<td>F</td>
<td>WP2 and WP3</td>
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<tr>
<td>Xin Zhang (50%)</td>
<td>Chinese</td>
<td>01.09.15–</td>
<td>M</td>
<td>WP6</td>
</tr>
<tr>
<td>Wenche Telle (25%)</td>
<td>Norwegian</td>
<td>01.01.2012–01.09.16</td>
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<td>WP4</td>
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<tr>
<td>Teresa Capelinska (30%)</td>
<td>Norwegian</td>
<td>01.01.2013–</td>
<td>F</td>
<td>WP4/ WP6</td>
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<tr>
<td>Daniela Dulgheriu (25%)</td>
<td>Romanian</td>
<td>01.18.2015–</td>
<td>F</td>
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ADMINISTRATIVE PERSONNEL WITH FINANCIAL SUPPORT FROM THE CENTRE BUDGET

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<tr>
<td>Frank Nilsen</td>
<td>Norwegian</td>
<td>01.09.11–31.08.19</td>
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<td>Centre Leader</td>
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<tr>
<td>Ingunn Wergeland</td>
<td>Norwegian</td>
<td>01.01.12–31.12.16</td>
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<td>Centre Coordinator</td>
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SLRC Publications

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<tr>
<th>No</th>
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<tr>
<td>1</td>
<td>Krasnov A, Wesmajer Xi, Bieland M, Hatlen B, Afanasiev S, Staniko Skugor. Sexual maturation and administration of 17ß-estradiol and testosterone induce complex gene expression changes in skin and increase resistance of Atlantic salmon to ectoparasite salmon louse. General and Comparative Endocrinology; Volume 212, 1 February 2015, Pages 34–43.</td>
<td>EWOS, NMBU</td>
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<tr>
<td>5</td>
<td>Helgesen KO, Aaen SM, Romstad H, Horsberg TE. First report of reduced sensitivity towards hydrogen peroxide found in the salmon louse (Lepeophtheirus salmonis) in Norway. Aquaculture Reports; Volume 1, May 2015, Pages 37-42.</td>
<td>NMBU</td>
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<tr>
<td>6</td>
<td>Bravo S, Silva MT, Agusti C, Sambral K, Horsberg TE. The effect of chemotherapeutic drugs used to control sea lice on the hatching viability of egg strings from Caligus rogercresseyi. Aquaculture; volume 443, 1 June 2015, Pages 77-83.</td>
<td>NMBU</td>
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<td>7</td>
<td>Kaur K, Bakke MJ, Nilsen F, Horsberg TE. Identification and molecular characterization of two acetylcholinesterases from the salmon louse, Lepeophtheirus salmonis. PLOS ONE; May 04, 2015</td>
<td>NMBU, UB</td>
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<td>8</td>
<td>Kaur K, Helgesen KO, Bakke MJ, Horsberg TE. Mechanism behind Resistance against the Organophosphate Azamethiphos in Salmon Louse (Lepeophtheirus salmonis). PLOS ONE; 20 Apr 2015, 10(4):e0124220.</td>
<td>NMBU</td>
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<td>9</td>
<td>Tz Chun Guo, Amr Ahmed Abdelrahim Gamil, Melanie Koenig, Øystein Evensen. Sequence Analysis and Identification of New Isoform of EP4 Receptors in Different Atlantic Salmon Tissues (Salmo salar L.) and Its Role in PGE2 Induced Immunomodulation In Vitro. PLOS ONE; April 2, 2015</td>
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The total activity for the SLRC in 2015 was 28 005 mill NOK compared to a budget if 29 168 mill NOK. Unused funding from RCN and the industrial partners are transferred to future periods.
### Publications 2013

<table>
<thead>
<tr>
<th>No</th>
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<tbody>
<tr>
<td>1</td>
<td>Torissen O, Jones S, Asche F, Guttormsen A, Skilbrei O, Nilsen F, Horsberg TE, Jackson D.</td>
<td>NHH, UB</td>
<td></td>
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<tr>
<td>2</td>
<td>Helgesen KO, Horsberg TE</td>
<td>Single-dose field bioassay for sensitivity testing in sea lice, <em>Lepeophtheirus salmonis</em>: development of a rapid diagnostic tool.</td>
<td>NHH</td>
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<tr>
<td>3</td>
<td>Helgesen KO, Horsberg TE</td>
<td>Influence of different materials on the concentration of delousing agents in sea water during bioassays.</td>
<td>NHH</td>
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<td>4</td>
<td>Lars A. Hamre, Christiane Eichner, Christoph Marlowe A, Caipang, Suzie T. Dalvin, James E. Bron, Frank Nilsen, Geoff Boxshall, Rasmus Skern-Mauritzen</td>
<td>UBB, IWR</td>
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<tr>
<td>5</td>
<td>Per G. Espedal, Kevin A. Glover, Tor E. Horsberg, Frank Nilsen</td>
<td>Emamectin benzoate resistance and fitness in laboratory reared salmon lice (Lepeophtheirus salmonis)</td>
<td>UBB, MA/ NHH</td>
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<tr>
<td>6</td>
<td>Oelkers K, Vike S., Duessus H., Gonzalez J., Wadsworth S., Nylund A.</td>
<td>Caligus rogercresseyi as a potential vector for transmission of Infectious Anaemia (ISA) virus in Chile.</td>
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### Publications 2012

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<td>1</td>
<td>Mennerat, A., Hamre, L., Ebert, D., Nilsen, F., Davidova, M. and Skorping, A.</td>
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<td>2</td>
<td>Krasnov, A., Skugor, S., Todorcevic, M., Glover, K.A. and Nilsen, F.</td>
<td>UB, NVH</td>
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<td>Dalvin, S., Nilsen, F., Skern-Mauritzen, R.</td>
<td>IMR, UB</td>
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<td>5</td>
<td>Skern-Mauritzen, R., Malde, K., Bonnier, F., Nilsen, F., Jonassen, I., Reinhardt, R., Koop, B., Dalvin, S., Mahle, S., Kongshaug, H., Glover, K.</td>
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<td>Nilsen, F.</td>
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### Publications 2011

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<tr>
<td>1</td>
<td>Nilsen, F.</td>
<td>Emamectin benzoate resistance and fitness in laboratory reared salmon lice (Lepeophtheirus salmonis)</td>
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