ANNUAL REPORT 2014
SEA LICE RESEARCH CENTRE
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 be over €300 million and the aquaculture industry relies heavily on a few chemotherapeutants for lice control. Emerging resistance development to these drugs increases the necessity to develop new treatment methods (biological, prophylactic and drugs) and tools to avoid increased loss due to sea lice and to ensure a sustainable salmon farming industry in the future.

The research conducted at the centre will focus on methods and tools to facilitate development of new drugs, 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.

Sea Lice Research Centre (SLRC) consisting of the leading scientists within the field together with the major industrial players will 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 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

The Sea Lice Research Centre aims at becoming a world leader for research on 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 (WP 1)
- Anti-attachment diets (WP 2)
- Immune controls (specific & nonspecific) (WP 3, WP 4)
- RNAi gene techniques for research tool development and future controls (WP 4)
- In depth knowledge of the molecular biology of growth, reproduction and endocrine systems in sea lice (WP 4)
- Annotated genome sequence linked into an integrated database containing experimental data (WP 5, LiceBase)
- Updated microarray and other molecular tools (WP 3, 4, and 5)
- Larval detection and assessment techniques (WP 4)
- Sea lice facility (naïve lice population, challenge facility, etc) (WP 6, LiceLab)
- Development of true integrated pest management techniques for industry (Part V)
SUMMARY – BY THE CENTRE DIRECTOR

The Annual Report for 2014 marks the end of the first three years of SLRC and a major task during the fall has been to conduct the self-evaluation as a part of the mid-term evaluation of the centre. A critical examination of our activity is a useful exercise in order to optimize centre performance and to make sure the resource is used as good as possible for a CRI. The Research Council has set up a list of success criteria for centres like SLRC that covers a broad specter of issues. To get the full effect from our consortium it is crucial that we achieve synergies and utilize the broad expertise present in SLRC across both WPs and partners. Obviously, this has been a main concern for the centre during the first years of activity and at the entrance of 2015 the SLRC consortium is now stronger than ever.

There is a significant increase in the number of published or accepted per-review articles for 2014. It has been a focus in SLRC during the year to make sure that results are transformed into scientific publications. For our PhD-students this is a very important part of the training and most of them are now involved in one or more articles. The publications can also be used as a tool to see that we collaborate both within the SLRC and also with other research groups outside the centre (nationally and/or internationally). The publication list from SLRC has publications with authors from two and more WPs as well as national and international co-authors. SLRC scientists are partners in a recently funded Horizon2020 project with more than 25 partners. This project (ParaFishControl) will start up early in 2015 and will be an important addition to our international collaboration.

The Norwegian Food Safety Authorities (NFSA) has been invited to write about sea lice status in this annual report. A major concern is related to dispersal of salmon louse where medicines have a reduced or no effect after treatment. Since use of medicine has been the most important tool to achieve low lice levels in salmon farms, increased frequency of resistant lice is a major concern. Experience has shown that both bath and oral treatment is necessary to achieve good control throughout the marine production cycle. In addition, detailed knowledge about salmon louse biology and host parasite interaction is an important tool to use for management of salmon louse both at farm levels as well as for the authority. Knowledge generated in SLRC is highly relevant for this and it is important for SLRC scientists to make sure that our achievements are communicate broad, both nationally and internationally. In February, the minister of Fisheries asked if she could visit SLRC for a meeting to be updated on our progress and also asked for our views on the current salmon louse situation. This shows the relevance and importance of the centre for national authorities.

A total of five patents have now been filed as a result of the research within SLRC in close collaboration between the academic and industrial partners. This demonstrates that research conducted within SLRC is innovative and relevant for the industrial partners. It also demonstrates that there are close and active collaboration between industry and academia, which is one of the main tasks for a CRI. It is expected that more innovative results will be taken further for commercialization in the year to come.

Frank Nilsen
Director SLRC
Summary – by the Centre Director

Sea Lice and farming industry in 2014
Paul Negård Norwegian Food Safety Authority

There are several reasons to keep low sea lice levels in fish farms. Norway has an international responsibility to protect biological diversity of wild Atlantic salmon populations and this requires low levels of sea lice in the aquaculture industry. At the same time the risk of developing reduced sensitivity and resistance to drugs shall be kept as low as possible, and to ensure good fish welfare at all times. Balancing these, often conflicting, goals is a very difficult exercise.

In 2014 the sea temperature (at 3m’s depth) has been 0.5–2°C over the last 5 years average. This has contributed to a more demanding sea lice situation at farming sites. In addition, the year started with some heavy winter storms along the coast, which made it difficult to control sea lice levels in early spring.

The coordinated spring treatment (mainly in April) was regarded a success, and the Institute of Marine Research (IMR) concluded that the wild salmon smolt essentially had migrated safely out to open sea. More farms chose to slaughter the fish (prematurely) prior to spring treatment instead of doing another medication. During late spring and summer farms in different regions experienced significant settlement of sea lice short time after treatment indicating high infection pressure particularly from June and onwards.

Based on the prospects early in 2014 the Norwegian Food Safety Authority (NFSA) was very specific about expectations to the farming industry – that they would increase supervision and pay special attention to responsible use of drugs, internal control procedures/systems in the companies and, if necessary, increase the use of legal slaughtering orders. In many cases the companies have therefore taken proper actions and measures, making it unnecessary for the NFSA to increase the use of sanctions.

In late June the IMR alerted the NFSA of high levels of sea lice on the wild sea trout in large parts of western Norway. The sea lice level on sea trout increased throughout summer and IMR has reason to believe that sea trout populations in large parts of southern and middle Norway have been affected by sea lice this summer and autumn. In the same period the average sea lice level in the fish farms was at fairly low levels in most farms, i.e. well within the legal limit (on average 8.8% of the farms over the limit per week.) In the present situation where the fish farmers depend on fighting sea lice with drugs, it is obvious that keeping low sea lice levels comes at a high cost. In 2014 there have been an increased number of medical treatments, especially with Slice (emamectin benzoate) and hydrogen peroxide, mostly in western Norway (Hordaland County). The industry has to use drugs in spite of lower expected treatment efficacy.

During the last 5 years a continuous development of reduced drug sensitivity, resistance (according to bioassay results) for nearly all active drug compounds used have been observed. The flubenzurones is still effective, but are used only to a limited extent due to negative environmental effects on wild crustaceans. The reduced treatment effect is partly compensated by using various combinations of drugs, increasing the concentration and longer treatment time. In many cases these treatments are off label and an increase in episodes of high mortalities under and/or after treatments have been reported (especially with hydrogen peroxide). The NFSA is worried about fish welfare if this development continues.

The development of reduced sensitivity and efficacy of the anti-sea lice drugs has been the most serious concern for the NFSA the last couple of few years. The industry’s RD-activity on non-drug methods for controlling sea lice is extensive, but with the exception of cleaner fish, these methods are still at a small scale field test phase and have not yet reached a level with any measurable impact.
ORGANISATION OF SLRC

SLRC has both academic and industrial partners outside Bergen, but the main scientific activity in the centre is located in Bergen and Oslo. SLRC personnel working at IMR and UiB are co-located in the SLRC facilities at UiB at Marineholmen. From the start of SLRC, the partners have focused on communication and facilitating arenas for collaboration. As the centre has finalized the third year of operation, we think the right frames for interaction has been established.

Partners

From 2014, the Norwegian School of Veterinary Science merged with UMB to the Norwegian University of Life Sciences (NMBU). All the rights and obligations in SLRC are transferred from NVH to NMBU. A planned sale of Novartis Animal Health AG to Ely Lilly&Co is expected to be finalized in the beginning of 2015. The industrial partners are complementary to each other and linked together by the academic partners. The 8 SLRC partners are:

- **University of Bergen** 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 SLRC. Senior scientists within biology, molecular biology and bioinformatics use their knowledge in SLRC. The main wet-lab activities take place at UiB, where broodstocks of lice strains are kept. PhDs and Postdocs are educated within the center.

- **The Norwegian University of Life Sciences (NMBU)** is represented in 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 SLRC. This partner is responsible for WP1 and WP3 and has close connections to WP2 and 4. Until now, the main cooperating partners have been PatoGen Analyse, Novartis, EWOS and UiB.

- **Institute of Marine Research** is represented in 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 SLRC with long history of sea lice research. Scientists are based in Bergen and Dirdal where research facilities have been expanded the last years. In SLRC, development of compounds that reduce the settlement and survival of lice will be in focus. EWOS Innovation is the leader of WP2 and is cooperating with NVH 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 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 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 SLRC. PatoGen is mainly involved in WP1 and WP4, and collaborate with the partners UiB, NVH, Marine Harvest ASA, Lerøy Seafood Group ASA and Novartis Animal Health AG.

- **Novartis Animal Health AG** develops and commercializes leading animal treatments that meet the needs of pet owners, farmers and veterinarians. Both the Aqua Health part at Prince Edward Island, Canada, and the Animal parasite unit in Switzerland take part in the SLRC work.

- **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 SLRC.

Collaboration between partners

Combining the partners’ knowledge and expertise is a key factor to achieve the goals for 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 to innovation and development of new products and methodology. Results from scientific activity in SLRC during 2014 have led to innovative ideas and pre-projects.

As a result of previously findings and product development by partners in SLRC, a joint collaborative project funded by FHF started at the end of 2014. UiB is coordinating the project “Merdvariasjon genetisk resistens” where PatoGen Analyse, Marine Harvest and Lerøy Seafood Group are SLRC partners, in addition to Grieg Seafood AS. The aim of the project is to better understand the dispersal of sea lice and to identify improved solutions for the situation related to sea lice within the farming industry.
Several of the projects run in 2014 had people from different WPs and from both Oslo and Bergen involved. To make sure we obtain synergies from the different areas of expertise within the SLRC inter-WP projects has been an important goal when building the centre. There are several scientific publications on the way now that show the results of these inter WP projects and we anticipate more of these in the future. Mobility of personnel between partners is an important tool to secure collaboration, both between industrial and scientific partners in SLRC, but also between the scientific environments in Bergen and Oslo.

Two more patent applications submitted in 2014 is an excellent example of results from innovative collaboration between partners in SLRC. The applications are sent from EWOS Innovation AS and PatoGen Analyse AS, and both are results from collaboration with the scientists at NMBU.

Management of the SLRC

As host for SLRC, UiB is responsible for the coordination of all activities in the centre. The day to day management is carried out by 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 2014 there have been several replacements in the Board.

SLRC Board Members in 2014:
- Harald Sveier, Lerøy Seafood Group ASA
- Ragna Heggebo, EWOS Innovation AS
- Neil Robertson, Novartis Animal Health AG
- Marit Solberg/Olav Breck, Marine Harvest ASA
- Vidar Aspehaug, PatoGen Analyse AS
- Karin Kroon Boxapen, Institute of Marine Research
- Lise Øvreås, University of Bergen
- Mona Aleksandersen, Norwegian University of Life Sciences
- Audun Wiborg – Chair of the board

The leaders of the WPs create the Leader Group of SLRC. The centre leader and the WP-leaders have a responsibility to ensure that the on-going activities in the WPs are coordinated...
to utilize each other’s expertise. The SLRC leader group is an important arena to work for the best possible results in SLRC, and is responsible for scientific reports to the board.

Members of Scientific Advisory Board (SAB) for 2014 are:
• Dr Ian Denholm, Rothamsted Research/University of Hertfordshire
• Professor Chris Secombes, University of Aberdeen

The main focus for SAB is guidance to future work on basis of the SLRC project description. In 2014 there has not been any meeting between the SLRC and the SAB members, but communication has mostly been made through emails.

Sea Lice Arrangements in 2014
Two workshops for SLRC personnel have been arranged during the year: 8–9 April at Finse 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. Detailed scientific presentations were given by PhD-students and postdocs carrying out the research. To share knowledge is an important tool for securing the dynamic work in SLRC and this meeting is an important facilitator to update partners on scientific progress. 26-27 August at Espegrend, Bergen, where the main focus was related to the mid-term evaluation and to discuss new areas for collaboration.

Sea Lice Day 2014 was arranged 27 August at UiB as an update on selected research in SLRC. All the partners and personnel working with SLRC issues were represented at the meeting. In connection with the development of the 2013 work plan and the revised Work Plan for the centre, two meetings between the WP-leaders and industrial partners have been arranged to facilitate industrial influence on the SLRC activity.

3 Board Meetings have been arranged in 2014. The SLRC board has decided to arrange the meetings at the different partner institutions. This is a way to learn more about the collaborating partners and strengthen the SLRC consortium. The Board Meetings in 2014 have been arranged at Patogen Analyse (Ålesund), UiB and Marine Harvest (Bergen).

Sea Lice solutions, seen from a Salmon farmer
Olav Breck, Group Manager R&D, Marine Harvest ASA

The lice situation in Norway in 2014, in terms of reported levels, has not changed substantially compared with previous years. However, the control of lice now involves greater challenges and costs. Multiresistant lice populations severely limit treatment options and in many areas bath treatment with H2O2 or oral treatment with chitin synthesis inhibitors are the only options available. In addition, with an increased incidence of reduced sensitivity to H2O2, the use of chitin synthesis inhibitors and/or non-medicinal control options (including freshwater) are the only tools to deal with the situation.

Development and use of non-medicinal control options has increased dramatically, and in particular biological control with cleanerfish has been implemented more broadly and along the entire coast. Several companies are now investing in the farming of lumpfish and wrasse and we see more and more good examples where lice can be held under the maximum limits throughout the production cycle using cleanerfish. However, there remain challenges in several areas particularly related to the second year of production, when infestation can become so large that cleanerfish are unable to keep pace. At the same time, supply of cleanerfish is still restricted in many places. To reduce infestation one must adopt methods that either shield the cages against infective copepodids in the upper water layers (skirt) or which hold the salmon deeper in the net (submerged light and/or feeding, use of snorkel). In many cases, farmers combine such methods and apply integrated concepts.

There is currently a comprehensive development of various types of shields and other technologies to control lice. Future successful control of lice must be built on an industry that is equipped to keep lice levels under control, thereby eliminating periodic large infestations that cannot be handled by cleanerfish and which will require frequent application of other control methods. To achieve this, each company must be diligent and apply a thorough surveillance of the situation and be adequately prepared to keep control on their own sites. Recent research indicates that a large proportion of the lice pressure on a site stems from the site itself and we believe that future control strategies must be based on intervention at lower levels of adult female lice at the cage level, to prevent self-infection and infection on surrounding sites.

The salmon farming industry is dependent on the SLRC making breakthroughs on new methods to control lice. Even although one may not get sufficient protection from a single method alone (for example a vaccine or a functional feed), used in combination and in an integrated manner these individual methods will still be valuable for the industry.
SCIENTIFIC ACTIVITIES AND RESULTS 2014

Work Package 1: Chemotherapy and resistance

Principle Investigator: Tor Einar Horsberg, NMBU

General introduction
This work package has two parts:

1. To explore possible new treatment methods and chemicals
   Screening of new chemicals for potential efficacy against salmon lice. The last chemical 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 industrial partners.

2. To develop robust assay methods for resistance testing against chemotherapyants
   Resistance development in salmon lice against chemicals used today. Resistance has been a major problem for salmon lice control in Norway since 2008, and a sporadic problem earlier. 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 industrial partners.

The activity in the screening part of the work package (WP1.1.) has in 2014 been to finalize in vitro test methods and testing of a series of model chemicals using these protocols. In addition, work aiming at closing more of the salmon lice life cycle without using live fish has been initiated. The activity in the resistance part (WP1.2.) has been development of discoveries made in 2012, 2013 and 2014 into suitable screening assays for resistance, and a search for new mechanisms.

The browser for the salmon louse genome (WP5) has become an increasingly important tool for WP 1 in 2014. A sea water wet-lab facilities have been rented from NIVA at the location Solbergstrand, Drøbak, 40km from Oslo to get access to live lice at a daily basis.

WP 1.1.: In 2012, a protocol for hatching of egg strings was developed and standardized. In 2013, a protocol for efficacy testing on preadult parasites was standardized for screening, both for short-term exposure (30 minutes) and longer term exposure (24 hours). In 2014, a protocol for efficacy testing of chemicals on the molting processes in L. salmonis was standardized. These three protocols have been used to study the effect of 27 chemicals on the hatching of egg strings and the survival of preadult parasites. The selection of chemicals was done according to the IRAC mode of action classification (www.irac-online.org/teams/mode-of-action). 27 candidate chemicals from 21 IRAC groups were tested in all assays.

Table 1.1: Model substances screened for efficacy against salmon lice in a hatching assay, a molting assay and a direct toxicity assay. The substances are grouped according to the IRAC classification scheme for modes of action.

All substances were tested on egg strings, nauplii or preadult parasites with a standard exposure of 50mg per liter for 30 minutes to mimic bath treatments. Preadult parasites were also exposed for 5mg per liter for 24 hours to mimic oral treatment of the fish.

In the hatching assay, the hatching of egg-strings and development of viable copepodids was recorded for 7 days. In the molting assay, nauplii 1, 1 day old, were exposed to the chemical for 30 minutes, and the number developing into viable copepodids was recorded for 7 days. In the preadult test, the direct effect on viability of the parasites was recorded immediately after exposure, and after 24 hours. When an effect could be recorded, the
studies were repeated with lower concentrations until the lowest effective concentration was found. A similar protocol was used when the parasites were exposed for 24 hours, the effect being recorded immediately after the 24 hours exposure period.

The studies were almost completed in 2014, but some wrap-up experiments are still ongoing. Thus, the results are still being processed. The main findings so far are that almost all chemicals acting on the nicotinergic acetylcholin receptor had a direct toxic effect on nauplii and preadult parasites. Also, chemicals acting on GABA and/or glutamate gated chloride channels were very toxic for preadult parasites. Interestingly, some of the insect growth regulators were very toxic for preadults in the 24h assay. Almost none of the tested chemicals affected hatching of egg strings.

In 2014, a new project within WP 1.1 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. The first hurdle was to try to get copepodids to attach to an artificial substrate and molt into chalimus 1. Several artificial substrates have been tested. For one of these, copepodids were instantly attracted to it and held on. Feeding and initial development of the frontal filament could be seen after 6 days, but no permanent attachment or molting has been observed so far. The studies are continued in 2015.

**WP 1.2:** In 2012, a novel mutation leading to insensitivity of the enzyme acetylcholinesterase towards the organophosphate azamethiphos (Salmosan™) was found. The mutation changed a highly conserved amino acid in the acyl pocket of the enzyme to a different amino acid that partly blocks the entrance to the catalytic triade of the enzyme. In 2013, several validation exercises of the significance of this mutation were done. In 2014, this validation has been continued together with the industrial partner PatoGen Analyse AS, who has developed the discovery into a commercial screening assay. PatoGen filed a patent application for the finding with joint ownership between NMBU and PatoGen, and is now offering the assay to the salmon farming industry in Norway.

*Figure 1.1:* Recording of toxic effects on preadult parasites

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**Figure 1.2:** Study of acetylcholinesterase from the salmon lice.

The target gene for pyrethroids, the gene coding for the voltage-gated sodium channel in salmon lice, has been studied carefully in a search for possible mutations that can explain insensitivity towards these chemicals. One finding was that salmon lice possess three paralogues of this gene. Expression studies revealed that one of these, NaV 1.1 was substantially more expressed than the other two. NaV 1.1 was sequenced in seven pyrethroid-sensitive and pyrethroid-resistant strains. No mutations that can be associated with resistance have been found.

A putatively hydrogen peroxide-resistant salmon lice strain has been collected in the field, and cultured in the wet-lab. Bioassays have revealed that the sensitivity of this strain towards hydrogen peroxide was only \( \frac{1}{5} \) of that of a fully sensitive strain. Also the F1 offsprings showed the same level of tolerance towards hydrogen peroxide. Through enzymatic and molecular studies, the expression of a particular gene was identified as the cause for reduced sensitivity. The partner PatoGen has submitted a patent application for this discovery, and is in the process of validating it for use in a commercial screening assay for this mechanism. A high-throughput molecular screening assay will be launched by PatoGen in 2015. Due to the patent application, publication of this finding has been postponed to 2015.

A putatively emamectin-resistant strain has also been cultured in the wet-lab. The objective has been to cultivate a highly emamectin-resistant strain of salmon lice for later studies of mechanisms. To obtain fully resistant parasites, the fish carrying the parasites have been injected with emamectin benzoate. A search for mechanisms behind reduced sensitivity towards this treatment was initiated in 2014. Through a review of other physiological,
biochemical and molecular studies on the effect of avermectins on arthropods, a hand-full of potential candidate genes involved in the development of resistance have been identified. Expression studies and sequencing of these has been initiated, and will be continued in 2015.

In 2014, an associated project dealing with resistance in the Chilean parasite *Caligus rogercresseyi* was initiated. A number of samples were collected in seven Chilean salmon farms and subjected to selection with azamethiphos, deltamethrin, emamectin benzoate and hydrogen peroxide. The samples were brought back to Norway, and are currently studied for potential mechanisms behind resistance development in this species. The studies will be continued in 2015.

**Work Package 2: Anti-attachment**

**Principle Investigator:** Simon Wadsworth, EWOS Innovation AS

**Introduction**

Genus *Lepeophtheirus* is highly host-specific parasites and *L. salmonis* will only complete its life cycle on a narrow range of salmonid species. The sea lice are unable to effectively suppress the immune system of non-host species and if *L. salmonis* attempts to settle onto alternative fish species, the subsequent immune response may well be lethal. Thus correct identification of the host is essential for attachment and survival for the lice. Sea lice have advanced olfactory and contact chemoreceptors that are capable of accurate identification of species-specific host molecules (Mordue and Birkett, 2009). Sophisticated receptors have recently been characterized for both *L. salmonis* (SLRC, 2013) as well as *C. rogercresseyi* (Núñez-Acuña et al., 2014). It has been shown that sea lice exhibit a number of directional responses to the specific compounds present in salmon mucus, including isophorone and 6-methyl-5-hepten-2-one (Bailey et al., 2006). Conversely these positive responses are inhibited if odours such as 4-methylquinazoline and 2-aminoacetophenone from non-host species such as turbot *Psetta maxima* are detected (Bailey et al., 2006). Salmon specific compounds may potentially be masked by a range of vegetable based products. Masking effects of garlic, *Allium sativum*, rosemary, *Rosmarinus officinalis*, lavender, *Lavandula angustifolia*, bog myrtle, *Myrica gale* and isothiocianates derived from the Brassicaceae family have all shown to successfully interfere with arthropod host-location (Pickett et al., 2010).

The overall aim of work package 2 is to identify and assess the effect of a range of masking and repellent compounds that reduce the settlement and attachment of sea lice. These compounds will be developed into commercial feed products to provide additional tools for sea lice control. They will be implemented into an effective integrated pest management programme involving medicines, husbandry and feed management.

**The planned work for 2014**

*In vitro methods and assay techniques*  
- The *in vitro* model development to continue during 2014 in Norway and Chile  
- Main focus will be to develop a frontal filament model for both *L. salmonis* and *C. rogercresseyi*  
- Further assessment of gene response of lice within the *in vitro* systems.

*In vivo* assessment of masking compounds in-feed  
- Assessment of masking compound on reducing lice loadings post challenge  
- Determine fish gene responses during challenge (physiological and immune pathways)  
- Assess combinations of masking compounds  
- Determine lice gene responses exposed to functional components during challenge

**Molecular techniques**  
- Develop qPCR assays for lice genes involved in host identification and other physiological pathways

**1. In vitro systems (background)**

A vertical Y-tube assay (Figure 2.1) was used to study sea lice copepodid directional response (rheotaxis) and swimming activity to host odors. Salmon entrained seawater was added down one of the arms (shaded), compared to a seawater control. 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.
Figure 2.2: Index of preference of sea lice copepodids to host-signal, masked with different concentrations of plant based compounds B1, B2, B3 (polynomial fitting) using the Y tube assay. Similar results were observed with both *L. salmonis* and *C. rogercresseyi*. By using this model a series of compounds were screened at a range of doses. The most promising of these compounds were then taken forward for further testing.

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. rogercresseyi*) were able to detect the salmon host odour within the substrate and quickly deployed their frontal filament (Figure 2.3a). 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 (Figure 2.3b). Using this method, the most effective plant based products previously identified in the Y-tube assessment have been validated as potential host masking compounds. To date this model has been less successful for *L. salmonis* as this genus do not possess a pre-formed frontal filament and require a number of days for this 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. More stable cultures are currently being assessed in collaboration with WP1. Initial results appear promising.

Successful candidate products identified in the Y-tube and frontal filament model have been taken forward for *in vivo*, in feed challenge. Anti-attachment product 1 has been assessed in 6 in feed studies to date and has been effective at reducing sea lice up to 28% (Figure 2.4).

Microarray and specific qPCR analysis of the skin showed differences between gene up-regulation in salmon responses between the dietary groups. Higher levels of immune genes associated with Type 1 (Th1) were associated with the fish fed anti-attachment diets compared to controls. These included a range of cytokines (IL-8), chemokines (CCL-19), anti-microbial peptides (cathelecidin), acute phase proteins (complement-C3a) as well as extracellular matrix re-modeling proteins (MMP; wound healing). Infection by the sea lice also induced changes in the gene expression between the dietary groups. The pro-inflammatory *myeloperoxidase* (MPO) was significantly up-regulated post sea lice infection, in the fish fed the anti-attachment diets (Figure 2.5).

In addition to pro-inflammatory responses, over 50 interferon (IFN) related genes were induced by the anti-attachment diets. These are known to have important anti-viral activity as well as acting as receptors, intracellular activity and effector genes. A number of genes associated with iron metabolism were induced by the anti-attachment diets including golgi residing metallo-reductase STEAP4 and sero-transferrin-1. Within the fish fed anti-attachment diets a number of key genes were down regulated including makers for tissue remodeling and myofiber proteins. Fast myotomal muscle troponin-T1 had a 9-fold reduction in expression in test feed compared to controls.
In contrast the fish fed the control diets showed induction of a number of genes associated with Type 2 (Th2) immune responses. These included genes involved in tissue differentiation, re-modeling and extra-cellular matrix (TGFβ). Pre-apoptotic lymphocyte marker G1/G0 switch protein was induced in the control fed fish and this has previously been associated with increased susceptibility to sea lice. Leukolecin, a well know gene for anti-inflammatory Th2 pathways, was also upregulated in the controls. Sex hormone binding globulin beta glycoprotein was induced, increasing the regulation of steroid and hormonal metabolism.

Sea lice are highly effective at suppressing the immune system as well as diverting the remaining responses away from Type 1 to a more generalized systemic, Type 2 pathway. The diversion is thought to significantly increase susceptibility of Atlantic salmon to sea lice infection (Skugor et al., 2008; Sutherland et al., 2011; Tadiso et al., 2011; Braden et al., 2012; Krasnov et al., 2014; Fast, 2014). Maintaining overall immunity and more effective pathways represents a significant development in sea lice control.

The anti-viral defences of fish are closely tied to the Type 1 responses. When these are down-regulated and diverted by sea lice infection there is a profound increase in the susceptibility of the fish to viral diseases. Recent results have shown a 6-fold increase in mortality in fish pre-challenged with sea lice prior to an ISA challenge. Key anti-viral genes such as Mx were found to have been compromised by the sea lice infection prior to the viral infection (Brown et al., 2014). These effects were evident within commercial farming operations when large losses occur due to co-infection with sea lice and viral pathogens. In the current study fish fed the anti-attachment diets had consistently higher anti-viral defences, with over 50 interferons (IFN) related genes being induced, compared to controls. This protection was maintained pre-infection as well as during the challenge.

Further dose ranging studies were conducted using anti-attachment diet 1. There was a significant reduction of up to 22% in the levels of C. rogercresseyi observed on the fish fed test diets, compared to controls (Figure 2.6).

Figure 2.6: Levels of sea lice (C. rogercresseyi) on Atlantic salmon 12 days post-challenge. Fish were fed varying concentration of anti-attachment diet 1 for 21 days pre-challenge. A minimum of 30 fish were assessed per tank with 3 replicates per dietary group.

In this study lice were assessed for molecular responses to the host. A total of 9 genes were identified in C. rogercresseyi for ionotropic receptors, including the specialized glutamate ionotropic receptors (iGluR).

The functions of these genes were associated with receptor activities, such as transport channels and trans-membrane signalling. The expression of the receptor genes varied between the developmental stages of the sea lice. The receptor gene Ionotropic kainite receptor 2-like-(1) was highly expressed in the juvenile and attachment stages of sea lice. The specific qPCR results showed an increased gene expres-

sion of Ionotropic kainite receptor 2-like-(1) in the lice that had been exposed to fish fed anti attachment diets, with highest expression observed in the anti-attachment + immune stimulant (Figure 2.7).

Figure 2.7: Specific qPCR analysis of ionotropic kainite receptor 2 like-(1) gene in C. rogercresseyi. Expression levels were higher compared to non-exposed controls. Increased expression was also observed in lice exposed to fish fed higher concentrations of anti-attachment diet as well as anti-attachment + immune stimulant –

The anti-attachment-1 product will be commercialised by EWOS during 2015. Diets will be introduced into farming operations, within an integrated programme to support existing medicines and management techniques. The aim of the diets will be to reduce sea lice settlement and maintain fish populations below the requirement for treatment for as long as possible. Also to increase the interval between treatments, if an intervention with medicine is required. The anti-attachment diet will also be important in maintaining an effective immunity and reduce the risk of con-current infection of sea lice and viral pathogens.

Work Package 3: Immunomodulation of the host

Principle Investigator: Øystein Evesen, 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.

3.1 Evaluate in vitro the immunomodulatory effects of sea lice extracts in an Atlantic salmon-derived cell line (TO cells or SHK-1)

In 2012 and 2013, we carried out studies in Atlantic salmon-derived cell lines examining the effects of PGE2 as a leading candidate produced by sea lice to dampen the host inflammatory response. The findings were that PGE2 inhibited the induction of CXCL-10, CCL-4, IL-8 and IL-1α genes (results not shown) in a time dependent and cAMP independent manner. In 2014, our work has been mainly devoted to documenting these findings and pursuant to this, a manuscript has been submitted to Plos One (Guo et al., accepted).
3.2 Establish a method to identify proteins/components involved in host-parasite interaction, using a combined genomic/proteomic approach.

These activities included characterization expression of EP4 receptors in tissues and isolated cells of Atlantic salmon (sequence, expression, modulation). During the previous year (2013), we reported the generation of custom-made antibodies against the salmon EP4 receptor that were used for studies of expression in tissues. The techniques used in these investigations were both immunohistochemistry and real time PCR. Additional studies in 2014 revealed that EP4 receptors were found in melanomacrophages in the spleen (Fig. 3.1a). Furthermore, the receptors were associated with intra-hepatic monocyte-like cells (Fig. 3.1b), intra-epithelial lymphocytic cells and rodlet cells in the pyloric caeca (not shown) strengthening the view that Atlantic salmon EP4 receptors have an important immune functions. The findings will be published in 2015.

Fig. 3.1: EP4 receptors in the different tissues of Atlantic salmon. Immunohistochemistry staining of EP4 receptors (red stain) in a) spleen and b) liver.

Importance of PGE2 expression for viability of sea lice and infection of salmon

In 2013, we reported the use of a zebra fish model for studying in vivo inflammatory responses to EP4 with the aid of morpholino technology. Because of the cofounding effect observed with the technique, we decided to discontinue the studies and instead opted to carry our RNAi knock-down of phospholipase pathway in sea lice followed by infection of salmon. PGE synthase (PGES), a gene involved in the synthesis of PGE2 was knocked down in lice using RNAi technology. This work was done as a collaborative effort with WP4. The sea lice were then used to infect salmon and at specific time points, different tissues including spleen, head kidney, skin, gill, and fin from infected or uninfected salmon were sampled. The knock-down of PGES in the louse was successful.

The expression of inflammatory genes in these tissues was examined in comparison with that of fish infected with wild type sea lice. The anticipation was that knockdown of PGE2 in sea lice would reduce interference of the host immune response in infected fish. The results however showed that there was in general no significant difference between groups (Fig. 3.2) leading to the conclusion that other factors besides PGE2 may in fact be responsible for modulating host responses. On this premise, and also the results obtained from WP 4 suggested that knockdown of PGES did not influence sea lice biologically/morphologically. One interesting finding from the study was that the progression of infection involves an initial attachment of copepodids on the fins followed by migration to the dorsal part of the fish.

From the studies reported under 3.1 and 3.2 the understanding is that PGE2 possibly play a role in modulating immune responses following infection with copepodids (and possibly later stages) but it cannot be fully ascertain this is the main or most important cascade of events. Further studies referred above (3.2) raise questions as to the synthesis pathway of PGE and there are distinct possibilities that alternative routes exist that can also lead to the formation of other, yet unidentified compounds, that play a role in dampening immune responses upon infection. For these reasons, it has been decided to vacate these research topics for the remaining of the project period and focus on ways of modulating (and possibly strengthen) the responses of the host to infection through various feed interventions. Further interactions between sea lice and virus infection will also be subject to more detailed studies over the remaining of the project period. And finally, personnel under WP3 will also be involved in vaccine development studies, particularly assessment of immune responses to vaccination.

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

In 2013 we reported on the local inflammatory skin responses induced by sea lice infection at gene transcript level. These studies were completed and results were published during 2014 (Holm et al. 2015).

In collaboration with WP2, EWOS, the impact of phytochemicals on the control of salmon louse infection was studied. The aim was to investigate if Atlantic salmon fed anti attachment compounds would be better protected against lice infection through modulation of the host responses at the site of infection. Groups fed different levels of anti attachment...
compounds were challenged, lice were counted and the local immune response was assessed by 21k oligonucleotide microarray and qPCR. A reduction of 25% (number of counted sea lice) was observed. The results have been compiled, a manuscript has been written and final details are being prepared for submission in the beginning of 2015.

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

Principle Investigator: Rune Male, UiB

The activity in WP4 covers three main areas, **copepodid biology, reproduction & endo and exocrine systems**. The aim is to provide a detailed understanding of the sea lice biology and deliver novel targets for development of drugs and vaccines against the salmon louse.

A sustainable long-term prevention of sea lice will require use of integrated pest control. In such a strategy, it will be important to develop new treatment tools such as vaccines, host immune stimulants, parasite repellents and new pesticides that are designed to attack sea lice with minimum risks for the environment and consumer. Several of present day methods suffer from development of treatment resistant lice. Mapping of resistance mechanisms have helped in development of monitoring methods used for best choice of treatment strategies. Development of new sustainable treatment methods against sea lice depends on detailed knowledge and understanding of the organism. Such knowledge may point out targets for vaccine development, reveal bottlenecks in regulation of vital live processes of the parasite and disclose details in host-parasite interactions to be utilized to fight the parasite.

Develop general tools and resources

The sequencing and assembly of the Lepeophtheirus salmonis genome is finished and has been an invaluable tool for the progress in WP4. Annotation of the salmon louse genome is a very complex task that includes participants in WP4 and WP5, together with Ensembl and partners from the “Sea Lice Genome Consortium”. The genome of the salmon louse contains many genes with little or no resemblance to genes known from other organisms. These genes can be promising targets as they are highly specific to just the louse or to only copepods and thus will have no side-effects in fish and humans. Working with this group of genes has been focused on looking for genes with an expression profile at suitable locations and time. A number of interesting genes has been studied during 2014 and further work including production of test vaccines will continue in 2015.

Gene knock down studies in adult female lice using RNA interference (RNAi) is a well-established and powerful method for functional studies of genes in sea lice organized within WP6 for several years. Recently, researchers in WP4 and WP6 have developed a refined method for stable gene knock-down by RNA interference in larva of the salmon louse. This opens for high capacity RNAi studies at a much reduced cost.

An update of the salmon louse live cycle was published by researchers in WP6 and WP4 in 2013. This year, a detailed follow up study of growth of sea lice larva has provided the necessary tools for stage determination and early sex dimorphism.

Host recognition, chemosensory systems

Free-living non-feeding larva characterizes the first copepodid phase while parasites in the second stage have identified and settle on a host. The parasite clearly discriminates between host and non-host fishes, infecting salmonid fish only. After settlement, the copepodids will start feeding and at the same time actively interacting with the host immune system. Understanding how lice recognize the host and subsequent immune modulating mechanisms can open for new options for lice control.

Characterize the chemosensory system in salmon louse: Two groups of genes characterize the chemosensory system in Salmon louse. Firstly, the Ionotropic Receptors (IRs) that are associated with smell/taste perception in invertebrates. Since salmon louse lack typical chemoreceptors, IRs are consider 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), in invertebrates associated with chemoreception and are important in development, reproduction or behaviour.

Many IRs have been identified in salmon louse with peak expression levels during free-swimming, infectious copepodid stage clearly indicating the engagement of IRs in recognition process of specific host. IRs works in pairs of one co-receptor with one specific IR, to detect and process chemical signals. Experimental infection of fish using copepodids with reduced levels of co-receptors by RNAi treatment, have been performed with success and will be continued in 2015.

Five genes belonging to Serpentine GPCR chemoreceptors were identified in salmon louse, known to be involved in numerous processes in invertebrates: FMRFamide receptor (FMRFa-R), Sex-peptide Receptor (SP-R) and three Myosuppressin receptors (MS-R R1, 1b and 2).

RNAi experiments were performed for all genes on nauplia I stage. Knock-down of several Serpentine GPCR chemoreceptors caused high level of mortality in copepodids. The RNAi treated copepodids were used to infect fish. Knock-down of the Myosuppressin receptors gave low recovery (Figure 4.1) and for one also reproductive effects. Further study on Serpentine GPCR genes will be carry on in 2015.
Gene knock-down of five Serpentin GPCR chemoreceptors gave reduced survival except for FMRF-R (part A). The highest loss of lice was observed for the three MS-R gene transcripts, 70% fewer lice were recovered from these groups than in control group. Knock-down of LsMS-R1a produced females with no egg strings (part B) and no spermatophore deposition on the female lice.

Moulting and general growth

Ecdysone receptor: a key regulator of molting, development and growth: 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).

Down-regulation of LsEcR gene expression in female louse lead to damage of the subcuticular tissue as well as complete loss of egg production (Figure 4.2), and eggs failed to mature inside the genital segment. From this, it is apparent that EcR directly or indirectly affects a variety of biological processes and makes it a good target for pesticide development.

Moulting; exoskeleton degradation and synthesis. Chitin is a major component of the exoskeleton of the louse. In the molting process chitin from the old exoskeleton has to be degraded. The chitin degrading enzymes, chitinases, are interesting targets for pesticide development. Chitinases have a variety of physiological roles and are found in many organisms, not only chitin containing ones. Therefore carefully studying of different chitinases and their functions is critical when searching for a new drug target.

In the salmon louse genome, three chitinases with high similarity to chitinases found in other crustaceans and in insects were identified. The expression patterns differed between the chitinases during development, pointing to different functions. RNAi knock down of one of them gave deformed copepodids not able to infect fish (Figure 4.3).

Iron regulatory protein: Iron regulatory proteins (IRP) play crucial roles in cellular iron homeostasis by regulating the expression of several proteins involved in iron transport and storage. Two IRP 1 homologues (but no IRP 2 homologue) are expressed in salmon louse ovaries, oviducts and vitellogenic oocytes of adult females, at all developmental stages. Knock-down by RNA interference (RNAi) in preadult female had effect; lice produced less offspring than control lice, had decreased levels of transcripts involved in egg development.
and caused increased expression of a salmon louse Ferritin (LsFer). RNAi knockdown in nauplii did not give any effect on lice survival and development into the copepodid stage. Maybe because feeding on blood only becomes important for the parasite at a later stage. Ferritin is an intracellular iron storage protein in prokaryotes and eukaryotes, but a secreted form exists in insects and worms that acts as an iron transporter. Two ferritin-like genes were knocked down by RNAi in adult female lice. The knockdown-lice were unable to reproduce. The two ferritins will be tested as vaccine candidates in collaboration with Novartis. Two additional ferritins have been identified but were shown not to be essential for reproduction or early development of the organism. A manuscript on Ls ferritins is in preparation and planned to be submitted in spring 2015.

Sea lice feed on host blood and must be highly adapted to tolerate very high levels of iron. A total number of 14 possible vaccine candidates – all involved in haem or iron metabolism – have been identified in the salmon louse and knocked down by RNAi in preadult females and nauplii. Knockdown was experimentally confirmed in adults and copepodids, respectively, but no phenotypes were observed except for the mentioned ferritin genes.

**Reproduction; germ cell differentiation and maturation**

**Function of Nanos in germ cell differentiation:** Germ stem cells are set aside early in embryo development that later in development form the gonads together with somatic cells and differentiate to male and female gametes. Establishment of male and female sea lice is first visible at chalimus II stage, indicating sex determination to take place in chalimus I or even before. We have used Nanos to study germ cells development in salmon lice. Nanos protein binds selected mRNAs and block translation of proteins that are necessary in germ cells growth and/or differentiation. Sea lice have three Nanos variants, where one is mainly expressed in the Nauplius II stage. RNAi knock down of this Nanos variant in Nauplia larva resulted in adult animals with largely empty ovaries and morphologically disturbed testis, as if the germ cells was not present or non-functional (Figure 4.4). Further studies of Nanos in a cell culture system designed to see interaction between protein and RNA has revealed promising results.

**Vitellogenin and Lipophorin regulation, transport and uptake:** Developing oocytes in all oviparous animals store nutrients essential for independent development of the embryo. Large amounts of proteins and lipids are deposited into growing oocytes during vitellogenesis and serves as building blocks and energy source for the developing embryo. Two major hemolymph lipoproteins, Vitellogenin (Vit) and Lipophorin (Lp) are responsible for accumulation of proteins and lipids into growing oocytes. During oogenesis, Vit is the major egg yolk protein (YP) while Lp (mammalian homolog apoB) carry most of the lipids to oocytes. Vit and Lp belong to a large lipid transfer protein (LLTP) gene family which also includes microsomal triglyceride transfer protein (MTP). In human, the assembly and secretion of apoB lipoproteins is facilitated by MTP. Similarly, the biogenesis of Vit in Xenopus laevis was found MTP-dependent. The function of MTP in connection with Vit and Lp assembly and secretion was studied in salmon lice. MTP RNAi knock down in sea lice resulted in no or abnormal egg strings and significant mortality of copepodids after hatching.

Lipophorin receptor (LpR) is involved in incorporation of Lp into the growing oocytes for their successful maturation and development. Salmon lice LpR has high structural similarities with insect and crustacean LpRs. LsLpR is highly expressed in adult female, genital segment and eggs (Figure 4.5).

**Immunomodulation, exocrine activity**

**Exocrine glands:** Exocrine glands produce and secrete proteins, mucus or pheromones that end up inside the body away from the gland, or on the body surface, via ducts (Figure 4.6). Exocrine glands can morphologically be divided into four types. One of the types is called tegumental glands, which are the most abundant glands in the salmon lice (Figure 4.7). They are present in the cephalothorax, genital segment, abdomen and appendages, have ducts that end up on both the dorsal and ventral side of the lice, differing in both size and appearance, and may be linked to different gene products. Studies have shown virus particles in salmon lice. Two probably lice-specific species were identified and shown to be present in exocrine glands of the lice but not in host fish (Figure 4.8).
Endocrine regulation

Processes such as sexual differentiation, germ cell maturation, molting and even general growth are highly influenced by gene regulators. Nuclear receptors are important gene regulators that may be highly activated by specific ligands (hormones), chemical signaling molecules that operate at very low concentrations. The salmon louse has more than 20 different Nuclear receptors. Bioinformatic analysis suggests that several of the receptors may bind specific ligands, but besides EcR this remains to be verified experimentally. The hypothesis is that molting and even other maturation processes can be manipulated by carefully designed inhibitors or activators. Ecdyson receptor (EcR) is expected to be a major regulator in sea lice, involved in several mechanisms and has been subjected to detailed functional studies including RNAi together with several other selected nuclear receptors.

A general assay for testing of receptor activation has been developed. A ligand for EcR has been identified that activate the receptor at very low concentrations typical for hormone activation (Figure 4.6). EcR need a receptor partner for gene activation. The main partner is as expected a protein called USP, but others may interfere, explaining some of the complexity of EcR as a major regulator of different physiological processes. This in vitro assay may be further used to search for potential inhibitors of these receptors. In collaboration with researchers in WP1, methods have been established to quantify the steroid hormone level in different developmental stages of the salmon louse. The hormone level has been measured using Mass spectrometry and is important to fully understand the ecdysone hormone/receptor pathway in different developmental processes like molting. This work will continue in 2014.

Transport in cells: Retrograde transport is movement of proteins, vesicles or even organelles inward, away from the synapse and plasma membrane or between organelles, like transport from endosomes to the trans-Golgi network. Two members of the molecular machinery that is involved in this process were characterized in sea lice. Knock-down caused disturbed digestion, absence of egg strings, high mortality and failed development, pointing to the potential of such transporters as suitable target candidates for new sea lice pest control methods.

Towards the function of the *L. salmonis* Prostaglandin E2 synthase: Salmon lice are able to modulate the immune defense of its host. Prostaglandin E2 (PGE2), a common prostanoid in parasitic secretions, is known to be an immune modulator in higher vertebrates. PGE2 has been detected in excretory products of the salmon louse and PGE2 has been shown to inhibit the expression of some pro-inflammatory genes in the salmon. However it still remains to prove that louse produced PGE2 is the main modulator of the immune system of the salmon.
The prostaglandin E synthase is an enzyme responsible for the last step of PGE2 synthesis in vertebrates. In the genome of the salmon louse one gene similar to the PGE2-synthase is found. We characterized this gene by measuring its expression profile in different developmental stages and its distribution in the salmon louse tissue. In collaboration with WP3, the PGE2 synthase was knocked down by RNA interference in nauplia and an infection study with emerging copepods is currently being investigated.

Test of vaccine candidates
Selection, production and clinical tests of vaccine candidates represent a close collaboration between researchers in WP4 and WP6, Dr Mark Fast’s group at UPEI, Canada and Novartis. Candidate target proteins are selected based on expression pattern, biological function, expected toxicity and suitability for recombinant production, and then tested for potential as treatment target by RNAi knock down. Promising candidates may include intestinal proteins including receptors and proteins and peptides predicted from exocrine glands RNA sequences, lipases and selected proteases and protease inhibitors will also receive attention. Four candidate proteins are currently under recombinant production at Novartis and will be further evaluated in 2015.

**Cooperation in SLRC**

**Neil Robertson, European Commercial manager Novartis Aqua**

Novartis Animal Health (NAH) and the Sea Lice Research Centre (SLRC) share a common goal of successfully developing new solutions in sea louse control and to manage what is a significant problem for the salmon farming industry globally. In 2014, the collaboration between the SLRC partners and NAH was both intensified and solidified with the signing of a joint ownership agreement. NAH provided a range of compounds to be tested in Work Packages 1 and 4 in both in vitro and in vivo assays. The range included several 'Novartis Experimental Compounds' (NEC), as well as other compounds of interest. Further tranches of compounds will be provided by NAH in 2015. The effective control of diseases in farmed fish has paramount importance for the development of sustainable aquaculture practices and the discovery of new technologies for the prevention of parasite infections supports our interests as an animal health company. Our collaboration with SLRC scientists and access to LiceBase and RNAi knock down technology has been essential to our sea louse vaccine project and has improved our understanding in a number of key areas in terms of testing potential vaccine candidates. The SLRC’s unique and extensive wet lab capacity using single fish testing tanks will soon be utilized to test prototype vaccines. NAH believes the SLRC consortium is the key to develop short and long term solutions to ensure a sustainable salmon farming industry in the future, and Novartis Animal Health is proud to be a partner in this mission.

**Work Package 5: LiceBase**

**Principle Investigator: Inge Jonassen, UiB**

In the beginning of 2014, LiceBase was released as a stable resource to the Sea Lice Centre. LiceBase supports general bioinformatics needs within the Centre and has become a central point of entry for all the centre’s genomics data on sea lice. After the release of a production grade portal service, our main focus has been on adding new useful data and features. We have also strengthened the system by writing documentation making it more user friendly and convenient, based on feedback provided by our users. Initiatives have been taken to increase the usage and to improve the quality of experimental and genome annotation data.

**LiceBase portal**

The most important event for LiceBase in the last year was its roll-out as a stable production version under the domain LiceBase.org in March 2014. The first release included the official genome annotation from Ensembl, data from 14 RNA-seq gene expression experiments, and all annotated experiments from the previous test phase.

The functionality of LiceBase has further been extended and enhanced. The most important addition is a new graphical interface to all Blast programs. This allows for efficient searches against the salmon louse genome and related data. There are currently 15 protein and DNA Blast databases available including different salmon louse assemblies and ESTs, transcriptomes of the closely related species *Calanus finmarchicus* and *Caligus rogercresseyi*, and the public salmon assembly. That way, users can quickly find their genes of interest in the salmon louse genome and GenBank ESTs, detect orthologues in other copepods, or check samples for potential contamination with salmon sequences.

All sequences in LiceBase can be blasted directly using a link “Run Blast” without copy-pasting sequences.

**Cooperation in SLRC**

**Neil Robertson, European Commercial manager Novartis Aqua**

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The graphic Blast interface is very user friendly: for genomic hits, the overlapping genes, exons, and other features are shown in the Blast result page, allowing to conveniently eyeball the correct placement of primers or PCR-fragment. In addition, the Blast result page provides links back to the genome browser to inspect the placement of sequences. Newly added sequences can now be assigned to genomic coordinates by a curator with a single click.

SLRC and its collaborators have taken great efforts to provide a highly accurate genome assembly and annotation. However, it is well understood that no existing genome sequence or automatic annotation are completely free of errors.

For example, there are gaps in the genomic scaffolds, and in some cases the automated annotation process missed genes. In our opinion, establishing a policy for reporting and handling deviations is the key to resolve observed discrepancies when dealing with genes and genomes, and we are working in tight collaboration with the EBI and Ensembl on a joint strategy for quality assurance.

In WP5, we have worked proactively on providing tools to enable a community driven curation process through LiceBase. Only if any perceived problems are reported, we can take an effort to fix them. We have therefore implemented a reporting tool for genome errata in LiceBase.

We have also implemented the possibility to add functional annotation and synonyms to genes, to import and annotate novel sequences, and to define their genomic coordinates. Novel sequences can also be placed automatically via Blast. Based on the current status of reports, only singular cases were encountered where a real gene was fully missed by the Ensembl annotation.

The primary value of LiceBase as a portal is composed of its rich data content. As of December 2014, LiceBase contains over 100 annotated RNA-interference experiments. During the year, WP5 received 9 additional transcriptomes analyzed by partners within the Centre. Samples comprise different tissues, such as intestine, antenna, testis, feet, glands, ovaries, and three more samples of the parasitic copepodid stages. As the data has been integrated into LiceBase, it allows researchers to better understand the important transition from free-living to parasitic life-style.

In addition, comprehensive on-line documentation has been written as a structured reference covering the most common topics of sea lice genomics. SLRC is actively maintaining a Blog for news and announcements. For a complete overview of the most current developments around LiceBase, please visit https://licebase.org/blog

Analysis
To make gene-expression levels comparable across stages, all 23 new and old data sets were re-analyzed using a new more efficient and accurate approach for mapping RNA-seq reads and generate more reliable and comparable expression estimates. Data are visualized using bar graphs for each transcripts, strand-specific tracks of local coverage are also available in the genome browser for all samples.

With respect to functional annotation of genes in the genome, WP5 has made available additional data through LiceBase. We have analyzed and imported the following additional data-sets:

- all ESTs from GenBank and all previous in-house EST sequences
- two available transcriptome assemblies of *copepods* *C. finmarchicus* and *C. rogercresseyi*
- domain annotations and Gene Ontology (GO) assignments from InterProScan
- mapping of full GO terms to GO-slim assignments, GO-slims are reduced and simplified versions of the full gene ontology
- a KEGG pathway reconstruction from the KEGG Automated Annotation server
- all publications mentioning *L. salmonis*, *C. finmarchicus* and *C. rogercresseyi* are automatically imported from PubMed

By combining Gene Ontology (GO) annotations and gene expression data, we have carried out a GO enrichment analysis of tissue and stage specific gene-sets. The results are visualized using the external tool Revigo.

WP5 has taken the initiative to foster the use of LiceBase within the centre and with collaborators. Two introductory courses (in Oslo and Bergen) were organized. Approximately 40 participants were introduced to the concepts of a model organism database, and computational genomics in general. Special topics of interest that were covered in the courses were:

- introduction to genomes and genome annotation
- using LiceBase to find and annotate genes of interest
- using LiceBase to help design better experiments
- annotating RNA-interference experiments

The originally planned Ensembl workshop to introduce the genome and Ensembl tools to the centre and the general public has been postponed.
Work Package 6: LiceLab

Principle Investigators: Lars Hamre, UiB and Sussie Dalvin, IMR.

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 (established 2012). The facilities have capacity 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.

The activities in WP6 can be divided into four main activities
• 6.1 Sea lice lab facilities
• 6.2 Production of sea lice and experiments
• 6.3 RNAi screening
• 6.4 Production of lice and lice experiments (EWOS)

Sea lice lab facilities: maintenance, improvement and development

Wet labs at UiB and at IMR has been upgraded in 2014 and approved as GMO facilities. Lice lab at UiB is partially upgraded with a new water supply for single fish tanks allowing separate water supply/outlet to each individual tank. This makes it possible to directly infect fish whilst in the tanks and is necessary to evaluate test vaccines and to follow up further development of RNAi treated nauplii more efficiently. Following an evaluation in late 2014 the lice lab has now become an approved test facility for Novartis.

Figure 6.1: Single fish tank system with separate water supply/outlets for individual tanks.

LiceLab in Dirdal houses 16 fish tanks (500 liters) and a hatchery supporting both stagnant and continuous flow incubators.

Production of sea lice strains, experimental material and experiments serving ongoing research in the SLRC

One new laboratory strain of salmon louse was established and a total of nine strains were maintained in 2014. Material for in vitro experiments and RNA and DNA purification was produced and sampled for academic partners in Bergen and Oslo, serving about 28 researchers/PhD’s/master students with material for ongoing research.

Lice material and incubator capacity was also provided to master and PhD students working with salmon louse outside the centre at the Institute of Biology, UiB.

About 50,000 copepodids were produced for the Refugium project.

Copepodids from several strains were produced for the FHF project “Selection of resistance of sea lice (Lepeophtheirus salmonis) to organophosphate and pyrethroid by combined treatment methods”.

LiceLab also provided material, lab facilities and experimental capacity to work on iron metabolism in the PrevenT project and work on aquaporins in the AQUALUS project. Both projects are supported by the Norwegian Research Council.

Other in-vivo experiments (lice on fish)
• Evaluating the effect of ultrasound exposure on copepodids for Marine Harvest.
• PatogenAnalyse: “who dies experiments” in order to improve test for resistance.
• FHF project: “Ferskvannsbehandling mot lakselus – mekanismeforetælse”.
• Emamectin benzoate resistance mechanisms: breeding trial.

RNAi screening to evaluate 600 (100/yr) potential gene targets

Two types of RNAi screens were carried out: I) RNAi in preadult II females and II) RNAi in nauplius. RNAi in preadults was performed by injection of preadult II females, after which the lice were placed back on fish. At termination of experiments, 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, behavioural 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.
Evaluation of RNAi screens from 2014 is on-going and results on specific genes will be reported from WP4. An overview of all experiment and results obtained in 2014 is summarized in the table below.

<table>
<thead>
<tr>
<th>Year</th>
<th>Method</th>
<th>Total RNAi screen</th>
<th>Total gene targets</th>
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<td>41</td>
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<td></td>
<td>Preadult</td>
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<td>2012</td>
<td>Nauplius</td>
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<tr>
<td></td>
<td>Preadult</td>
<td>51</td>
<td>41</td>
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</table>

Table 6.1: RNAi screens in 2012–2014. Total RNAi screens reflect the total number of fragments injected or incubated. Total gene targets: this number shows how many different genes have been tested, i.e. total numbers minus controls and replicated experiments. Nauplius RNAi was not implemented before 2013.

RNAi experiments on nauplii and preadults were also performed in collaboration with international partners (University of Prince Edward Island, Canada and Fiskaaling, Faroe Island) by visiting post doc.

Production of lice and lice experiments (EWOS)
The hatchery capacity for producing infective stages of *L. salmonis* was increased during 2014. This reflects the increasing demand for copeopdids for both in vitro and in vivo challenges. The seawater configuration was upgraded to allow improved disinfection as well as security of supply for the facility. Additional experimental equipment was installed to run in vitro models that allow copepodids to feed and develop a frontal filament.

INTERNATIONAL COOPERATION

The research partners in SLRC are leading scientists within their field of work and have a large international network. The senior scientists have a long experience in collaboration with research-groups and companies worldwide, and are attractive partners in international collaborating projects. A major plan is to include the younger scientists in the existing networks, and to encourage them to expand and to create their own networks. Four of five industrial partners are world leading within their businesses, and have significant activities in many countries. For the industrial partners in SLRC, major parts of their activities take place in UK, US/Canada and in Chile. With joint interests, it is a natural result that both the industrial partners and the research partners in SLRC have a close collaboration with research institutions in these countries.

The partners participate in EU-projects within the latest Framework Program and in Horizon 2020. Two of the research partners have been engaged in different applications within the SFS-10-2015/2015 call (Societal Challenge 2): “Tackling disease related challenges and threats faced by European farmed aquatic animals.” One of the applications has been granted, and the project ParaFishControl with 25 partners throughout Europe will run for 5 years from 2015. The involvement in the Horizon2020 project will extend our international collaboration and further make SLRC visible in the international arena. Horizon2020 is an important platform for international collaboration and innovation, and we expect more applications where both the academic and industrial partners in SLRC will be participants.

There is a close collaboration between SLRC and the elixir.no, a nationally funded project that in 2014 became a node of the pan-European bioinformatics infrastructure (ESFRI-project).

Both researchers at different levels and students are interested in the SLRC field of work, and would like to visit the centre for short or long periods. In 2014 researchers from Canada, China and the Faroe Islands have worked as research fellows in SLRC. In addition, students from France and Mongolia have had 6–8 weeks traineeships in the centre; both through organized mobility programs as IAESTE, but also as a result of direct contact to the researchers in the centre. SLRC has excellent researchers and facilities that make the centre an interesting institution to visit and presentations at international conferences together with publications are the major tools for showing the quality of the SLRC and to attract new collaborating partners.

The other way of sharing the knowledge and quality of research in SLRC is outgoing mobility. In 2014, two postdocs have visited international cooperating partners for 4–9 weeks, and one of the Principal Investigators is currently a visiting research fellow at University of Stanford, School of Medicine.
RECRUITMENT

A new Postdoc has been hired at NMBU from August 2014; in addition, a PhD employed at Høgskulen in Ålesund and enrolled at UiB is working for SLRC. Furthermore, the positions in SLRC are stable and without any major delays. The research partners are actively searching for related research to increase the activities in SLRC, and new peripheral projects such as EU-projects will add more funding and personnel to SLRC.

All together five master students have finalized the master programme at UiB and NMBU in 2014, and four new students have been recruited for 2014/15.

There has been minor changes in the SRLC personnel during the year, and by the end of 2014 the balance between the genders is 47.7% females and 53.2% men, which is a minor increase in females compared to 2013.

The overall gender aspect in SLRC is in accordance with the strategy outlined in the project description and satisfies the requirements for female-male ratio. New recruitment is planned to be 50% females and 50% males. Not all the key personnel are working full time for SLRC.

SLRC COMMUNICATION AND DISSEMINATION ACTIVITIES

Participation in international and national conferences is an important part of our communication strategy and SLRC members presented research as invited key-note speakers, speakers and with poster presentations. Although participation at conferences related to fish health is where most scientists tend to go, SLRC members also attend more general conferences even as invited key-note speakers. At national level, industry, authorities, private and public organizations and society in general, are interested in sea lice research and news from SLRC. An example is that the current Minister of Fisheries visited SLRC in February 2014 to be updated on our progress and our view on the salmon louse situation. Information and results from SLRC are posted on the centre’s website www.slrc.no, but news posted through media and other public channels are important ways of communicating the research in SLRC.

SLRC delegation at Sea Lice 2014

The biannual scientific conference “Sea Lice” gathers the world’s leading experts in the various fields of sea lice related research – from engineering to informatics and, of course, biology. The 2014 edition was the 10th in a row, sealice2014.businesscatalyst.com and took place in Portland (Maine) with 198 participants.

The Sea Lice Research Centre sent a large delegation of senior scientists and PhD students from academia and industry to the conference, with focus on participation, communication and with the aim to be the main contributor to presentation of news and research results. The SLRC annual report 2013 was distributed at the conference.

This year’s program consisted of over 80 academic contributions. Four invited plenary talks provided more general overviews about the development of sea lice research and management. In addition, over 70 presentations on state-of-the-art knowledge from scientists, industrial actors and others involved with basic sea lice research and management were held. The 14 participating scientists and PhD-students from SLRC gave a total of 14 presentations, split between 7 of the 11 different themes at the conference. SLRC also contributed with 4 posters. In line with the activities of SLRC, a large proportion of genome-wide studies, such as transcriptomics studies were presented at sea Lice 2014, highlighting the importance of key resources such as the annotated genome sequence of _L. salmonis_ and LiceLab. Studies with the aim to create anti-sea lice vaccines were also presented.

Awards

Kiranpreet Kaur, postdoc at the Sea Lice Research Centre, was the winner of the prestigious Young Scientist Award at the Sea Lice 2014 conference in Portland, Maine in September 2014. Kiran Kaur had studied the mechanisms behind resistance development against a commonly used treatment, azamethiphos. The jury, an independent panel of expert judges from industry and academia, emphasized that the results of the studies could have immediate practical applications, that they were carefully conducted and that she had used many advanced experimental techniques in her studies. Kiran Kaur was invited to the Novartis experimental station in St. Aubin, Switzerland, to meet with Novartis scientists, learn about their work regarding screening of compounds for antiparasitic effect and experimental methods that could be utilized in upcoming studies. www.worldfishing.net/news101/industry-news/young-scientist-award-winner-announced
Helle Holm recently won the Best Poster prize at the Inaugural meeting of the UK and Ireland branches of the European Association of Fish Pathologists at the University of Keele, England. The audience showed keen interest in the multivariate statistics approach used by Helle Holm in her research of selective breeding as a tool to improve resistance to salmon louse in Atlantic salmon. The poster revealed for the first time that immune markers traditionally considered to be part of anti-viral responses are associated with protection against lice. This exciting finding has recently received very good reviews in Fish and Shellfish Immunology as well and will soon be available in the article entitled “Difference in skin immune responses to infection with salmon louse (*Lepeophtheirus salmonis*) in Atlantic salmon (*Salmo salar L.*) of families selected for resistance and susceptibility”, where Helle is the lead author.

Stand at Forskningsdagene 2014
Communication was the main theme for the National Science Week 2014, and SLRC had a 2-days stand at Bergen Science Fair in September where people could learn about sea lice. 1500 schoolchildren (11–12 yrs) visited the Science Fair the first day, and 6000 persons visited the various stands the second day. All SLRC people – participated at the arrangement either by planning or being at the stand for the two days. The Science Fair is an excellent possibility to communicate to the society what is going on in SLCR, in addition to give information on the parasite and the various problems it causes. Sea Lice has been focused on in various media during the spring/summer 2014, and people were interested what a centre as SLRC could do for both the farmed fish and the wild salmon and sea trout. The visitors could have a look at the sea lice trough different microscopes or an aquarium. Behavior of both male and female sea lice was shown on TV-screens, in addition to a close study of molting. Sea lice in different stages in life cycle were shown, and many of the visitors were surprised about the size and shape of the parasite. Especially children were thrilled when they could let a sea lice “taste” their skin/finger and understood that sea lice do not like humans but salmon and sea trout only!

The number of publications from SLRC in 2014 is close to the planned number, and most of the publications have been published with Open Access. A number of publications worked with in 2014 are waiting for acceptance, and is expected to be published in the beginning of 2015. The SLRC publications are cited a number of times, and shows the importance of the scientific work in the centre.

SLRC dissemination activities:
APPENDIX

Personnel Sea Lice Research Centre 2014

KEY PERSONELL

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<tr>
<th>Name</th>
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<td>Frank Nilsen</td>
<td>UiB</td>
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<td>Sussie Dalvin</td>
<td>IMR</td>
<td>WP4, WP6</td>
</tr>
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<td>Rune Male</td>
<td>UiB</td>
<td>WP4</td>
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<td>Tor Einar Hansberg</td>
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<td>Øystein Evensen</td>
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<td>Inge Jonassen</td>
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<td>Lars Hamre</td>
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<tr>
<td>Sindre Grotmol</td>
<td>UiB</td>
<td>WP4, WP6</td>
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<td>Christiane Eichner</td>
<td>UiB</td>
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<tr>
<td>Kevin Glover (Professor II)</td>
<td>UiB</td>
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VISITING RESEARCHERS

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<tr>
<td>Ming Li</td>
<td>UiB</td>
<td>Chinese</td>
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<td>Gunnvor Joensen</td>
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<td>Faroese</td>
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<td>Okechukwu Igboeli</td>
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POSTDOCTORAL RESEARCHERS WORKING ON PROJECTS IN SLRC WITH FINANCIAL SUPPORT FROM OTHER SOURCES

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<tr>
<td>Christine Triesse</td>
<td>German</td>
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<td>Jon Anders Slavang</td>
<td>Norwegian</td>
<td>01.01.12–25.03.15</td>
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<tr>
<td>Ceba Augustin-Ridaura</td>
<td>Spanish</td>
<td>01.07.14–30.06.16</td>
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<td>Christopher Haaves</td>
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PHD STUDENTS WITH FINANCIAL SUPPORT FROM THE CENTRE BUDGET

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<td>Mohammad T. Kahn</td>
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<td>Iwia Harasmacro</td>
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POSTDOCTORAL RESEARCHERS WORKING ON PROJECTS IN SLRC WITH FINANCIAL SUPPORT FROM OTHER SOURCES

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TECHNICIANS WITH FINANCIAL SUPPORT FROM THE CENTRE BUDGET

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<td>Lars Are Hamre</td>
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* Employed as researchers at Postdoctoral level
The total activity for SLRC in 2014 was 32,031 mill NOK compared to a budget of 29,231 mill NOK. Some of the industrial partners have been more involved in SLRC than planned for, and have generated more activity that budgeted for in 2014.

Unused funding from RCN and the industrial partners are transferred to the period 2015–2016.

SLRC Publications

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<tr>
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<td>Torissen O, Jones S, Asche F, Guttmansen A, Skilbrei O, Nilsen F, Horsberg TE, Jackson D.</td>
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<td>Salmon lice – impact on wild salmons and salmon aquaculture.</td>
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<td>J Fish Dis 2013, 36, 261-272</td>
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<td>3</td>
<td>Helgesen KO, Horsberg TE</td>
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<td>Influence of different materials on the concentration of delousing agents in sea water during bioassays.</td>
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<td>The Salmon Louse: <em>Lepeophtheirus salmonis</em> (Copepoda: Caligidae): Life Cycle Has Only Two Chalimus Stages.</td>
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<td>Emamectin benzoate resistance and fitness in laboratory reared salmon lice (<em>Lepeophtheirus salmonis</em>).</td>
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<td>Life history and virulence are linked in the ectoparasitic salmon louse <em>Lepeophtheirus salmonis</em>. Journal of Evolutionary Biol (5): 856-861. 25 May 2012</td>
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