Background of the Sea Lice Research Centre
– Centre for Research-based Innovation

Sea lice (Lepeophtheirus salmonis and Caligus spp.) are the major pathogens affecting global salmon farming industry and have a significant impact in many areas. The annual loss has recently been estimated to more than €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 world leading in 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
SUMMARY

The 2012 annual report describes the first full year of activity for Sea Lice Research Centre. Although the first months of the year were characterized by hiring PhD-students and postdocs the research activity has reached good speed and all WPs are on track according to the 2012 year plan. The scientific kick-off was arranged in February and we arranged a SLRC workshop in September to bring all members and partners up to date on our activity. Arrangements like this are also crucial in order to facilitate interactions between WPs located in Oslo and Bergen, and it serves as an important arena for the industrial partners to be up-dated on the scientific activity within the centre.

The developmental stages of *L. salmonis* were described in detail more than 20 years ago but circumstantial evidence has accumulated indicating that the life cycle has been incorrectly described. By direct observation on moulting of chalimus stages in our hatchery, SLRC scientists have contributed significantly to a study where the salmon louse life cycle is revised. These data are submitted to publication and reveals that there are only 8 instars in *L. salmonis*, similar to other copepod species.

The two infrastructure/resource WPs in SLRC (WP5 and WP6) have developed substantially during 2012. LiceBase is subject to testing for SLRC members and will represent a useful tool for the centre. In this context it is also relevant to mention that the *L. salmonis* genome assembly is finished and now subject to annotation by Ensembl. Both the assembly and the fully annotated genome will be extremely useful for SLRC on constituting a valuable starting point to increase the knowledge about salmon louse biology and to identify and evaluate new treatment targets. In addition, the Atlantic salmon genome assembly will soon be available and having both the host and parasite genome, and will enable a range of new studies and projects relevant for SLRC.

The majority of construction work in LiceLab has been completed while experimental work has been going on. A key element has been to initiate the RNAi screen in pre-adult and adult female lice. At the end of 2012 we finally had success in applying RNAi in the free-living lice larvae. Although the method will be further validated and refined during 2013 this opens up the opportunity to conduct large scale high through put RNAi screening in these developmental stages for identification of novel treatment targets. In addition, this opens up a complete new experimental approach where RNAi can be combined with host parasite interaction studies in parasitic copepodids and the chalimus stages.

SLRC is still organised as described in the project proposal. The nature of SFIs where research results will be explored for commercial utilisation together with the industrial partners means that the interaction between industry and academia will increase as more new data are produced. To increase the visibility of these combined efforts, we will define more clearly fundamental and applied research (i.e. industrial related research) in the WPs. This will simplify our structure and reports and it is anticipated that the WPs in the future will contain several applied sub-projects.

Obtaining additional funding is one of the success criteria for an SFI. In 2012 SLRC is partner in two new projects. One project is funded by the research council of the Faroe Island and aims at identifying hatching enzymes in the salmon louse. SLRC through WP4 and WP6 is involved in several aspects in this study and some of the experimental work will be carried out in Bergen. In a funded application managed by CIGENE at UMB, SLRC is partner in a HAVBRUK/BIOTEK2012 project. This project aims to further understand the mechanisms behind the heritable difference in salmon louse susceptibility observed among families and individuals of Atlantic salmon. SLRC is involved mainly through WP2, WP3, WP4 and WP6. The project will utilise state of the art functional genomic tools on both the host and parasite to characterise the response in high and low susceptible families. The HAVBRUK/BIOTEK2021 project will extend the collaboration in SLRC towards CIGENE which is the central Norwegian partner in the Atlantic salmon genome project. In addition, this project will create a strong link between WP3 and WP4/ WP6. In the coming years it will be important to obtain further additional funding, and if new project proposals are made as collaboration between 2 or more SLRC WPs this will facilitate interactions between SLRC scientists.

Since the new SFIs were announced by the Norwegian Research Council, expectations have been very high from many parties that the sea lice problem would be solved in a snap shot when SLRC was funded. Further emergence of resistance against the available medicines put demands on SLRC and others to contribute to a sustainable aquaculture industry with minimal negative environmental impact. Results obtained in WP1 on effects of H₂O₂-treatment shows that exposed egg strings do not hatch. This knowledge is highly relevant for the authorities since it shows that removing of egg strings post H₂O₂ treatment is not necessary. We anticipate that more of the achievements from 2012 will be translated into useful tools for the industry in 2013. It is expected that more publications will be submitted during 2013 based on results obtained in 2012 from all WPs.

Frank Nilsen
Centre Director
The SLRC has five industrial- and four academic partners, there has not been any change of partners in SLRC since the opening of the centre in 2011. The industrial partners are complementary to each other and linked together by the academic partners. SLRC partners are:

- University of Bergen (Host Institution)
- Institute of Marine Research
- Norwegian School of Veterinary Science
- UNI Research AS
- Lerøy Seafood Group ASA
- Patogen Analyse AS
- Novartis Animal Health AG
- Marine Harvest ASA
- EWOS Innovation AS
The overall decision making body is the SLRC board, where all the nine partners have one representative. The Board takes decisions on strategy, annual work plans, activities, budget and the organisation. The SLRC board is chaired by an independent board leader. Two of the board members have been changed during the year.

SLRC Board Members in 2012:
- Harald Sveier, Lerøy Seafood Group ASA
- Einar Wathne/Ragna Heggebø, EWOS Innovation AS
- Ian Thompson/Asmund Baklien, Novartis Animal Health AG
- Marit Solberg, Marine Harvest ASA
- Reidar Toresen, Institute of Marine Research
- Lise Øvreås, UNi Research AS
- Mona Aleksandersen, Norwegian School of Veterinary Science
- Kari Espolin Fladmark, University of Bergen
- Vidar Aspehaug, Patogen Analyse AS
- Audun Wiborg – Chair of the board

The scientific activity in SLRC is organized in 6 Work Packages (WPs) and the leaders of the WPs create the Leader group of SLRC. SLRC has both academic and industrial partners outside Bergen, and this has been emphasised when the “centre-feeling” in SLRC has been established. The University of Bergen is the host institution for SLRC, and all people located in the Bergen area are co-located at the facilities in the High technology Centre where both dry and wet-lab facilities are available. 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.

2012 is the first year with full activity for SLRC, and the collaboration between the academic and industrial partners has been a key issue during the year. The extent of collaboration will be variable during the lifetime of the centre and dependents on scientific achievements. As vacant positions have been filled, the scientific activity has increased during the last half of 2012, and was by the end of the year at full scale. The process of building the Centre as one unit has been a prioritized task for the SLRC management in 2012, and several joint activities have been organized to get an optimal start for the SLRC:

- Kick off meeting for SLRC was arranged in Bergen 15 February. 40 people from all the partner organisations attended the meeting where the overall research plans for SLRC and the partners were presented.
- Tour to one of Lerøy Seafood Group’s fish farms outside Bergen 26 March. The main purpose for the tour was to see how de-licing is conducted and an introduction into
modern salmon-farming. 23 persons from both Bergen and Oslo participated on the tour.

- Workshop at Espegrend Research Station 26–27 September was arranged as the first join meeting for all new PhD students and Postdocs in SLRC. The industrial partners were also represented at the Workshop, and Novartis gave a presentation of their worldwide activities.

Formal arrangements in 2012

- 3 Board Meetings have been arranged
- Site visit from RCN was arranged 25 September, where all the levels in the SLRC-organisation were represented

Establishment of routines and good communication between the administrative personnel at the different partner organisations is important for the management of SLRC. 2012 has been a test on how the planned rules and routines are functioning. Some adjustments have been made throughout the year and corrected plans for 2013 are implemented.

New projects between partners in SLRC and other organisations will be added to the centre. At the end of 2012, two new projects were accepted for funding, the activities are planned to start in 2013. Some of the industrial partners have signalled plans for extended activity within the frames of SLRC.
SCIENTIFIC ACTIVITIES
AND RESULTS

Work Package 1: Chemotherapy and resistance

In the workpackage, there has been a high activity in both sub-tasks in the period.

Novel chemotherapeutic agents

A pilot study has been conducted to explore the possibility of getting a sufficient treatment efficacy of a spray or short-term dip in a concentrated pyrethroid solution. The study was initiated because manual removal of parasites using high pressure water jets combined with soft brushes is under development as a non-chemical method for removal of salmon lice. Pilot studies have demonstrated that full efficacy of such a system is unlikely because of possibility of damaging the skin in the procedure. A combination with a short term spray or dip in a chemotherapeutic agent coupled with a recirculation system may provide better efficacy with minimal discharge of effluents. In the pilot test, it was demonstrated that high concentrations of cypermethrin were lethal to even pyrethroid-resistant preadults and adult parasites. The rape-seed oil formulation used in the test was not well suited due to its viscosity, but an aqueous microemulsion was effective. A formulation with thinner oil might have better efficacy and be easier to handle.

One important aim in this WP is to develop and validate protocols for efficacy registration of interventions on each step in the life-cycle of the parasite: Hatching of egg-stings, development into copepodites, infectivity of these copepodites, direct effect on chalimus- and preadult parasites, fertility of adults. Using this model, the total impact on parasite populations can be calculated better that just spot-checking the efficacy on a single developmental stage. In 2012, a protocol for hatching of egg-stings has been developed. The basis for the protocol is to split 6 egg-stings from different female lice into four pieces each and using one piece from each egg-string for each of the concentrations of an agent that is tested. The risk of non-fertilized egg-stings having a significant effect on the outcome of the study is thereby eliminated:

![Control Dose 1 Dose 2 Dose 3](image)

Figure 1.1: Egg strings from 6 different parasites are each cut in four pieces. One piece from each egg string is incubated with different concentration of the chemical to be tested. The risk that unfertilized egg strings are interfering with the results is thereby eliminated.

Studies on controlled infections with a fixed number of copepodites on single fish have also been initiated. It has been difficult to avoid a substantial variance in the number of copepodites attaching to the fish. Different time series of exposure, point-exposure, belly-only exposure etc. has been tried, but with limited success. These tests will continue in 2013.

A laboratory study has been conducted to explore the hatching capability of egg-stings exposed to varying concentrations of hydrogen peroxide. Hydrogen peroxide is used in some areas as a chemotherapeutant against salmon lice, and concern has been expressed that the egg-stings from adult females released to the water after treatment could hatch. The study demonstrated that this is unlikely to happen. Concentrations substantially lower than the concentrations used in delousing procedures resulted in little or no hatching of the egg-stings, and none of the few nauplii that actually hatched reached the infective copepodite stage. These results were confirmed at normal treatment doses in a field study conducted in connection with a full-scale treatment with hydrogen peroxide. A manuscript is currently under preparation.

One of the aims in this task is to conduct a screening of a series of compounds from classes of insecticides with different modes of action. In co-operation with the partner Novartis, a list of 20 potential model substances has been prepared and small amounts of these have been purchased. The actual screening starts in 2013, first on direct effects on mobile stages, then on effects on hatching capabilities as these protocols are established. Promising compounds will be tested on chalimus stages and for a possible effect on the fertility of the parasites. When the controlled infection model is validated, this will be used to test the infectivity of copepodites.
Resistance

The first focus in this task has been to identify the mechanism behind resistance towards the organophosphate azamethiphos, validate the mechanism against in vivo sensitivity and biochemical activity, and develop rapid screening assays. A point mutation in one of the two genes coding for the target protein for this compound has been identified. This has been validated against sensitivity results, and the validation against biochemical activity is ongoing. The partner Patog en will use the results to develop a commercial assay for the mechanism that can be offered to the fish farming industry. A rapid and simple High Resolution Melt analysis for this change has been developed for laboratory use:

Figure 1.2: Genotyping of homozygote resistant, heterozygotes or homozygote sensitive parasites is done by High Resolution Melt (HRM) analysis. The figure illustrates results from parasites homozygote for the resistant type of the gene (blue), heterozygotes (green) and homozygote wildtype (red). Only samples with inconclusive clustering to one of the groups need to be sequenced.

The second focus has been to screen the gene coding for the voltage-gated sodium channels in salmon lice for possible mutations that can be associated with resistance towards pyrethroids. In earlier studies, one such mutation has been identified in salmon lice:

In samples collected recently, this mutation has not reappeared in resistant salmon lice strains, pointing towards other mechanisms also being responsible for resistance development. Larger parts of domain 2 and domain 3 are currently studied for possible mutations. These studies will be completed in 2013.

Work Package 2: Anti-attachment

Sea lice have advanced olfactory and contact chemoreceptors that are required for accurate identification of specific host molecules. Both Lepeophtheirus salmonis and Caligus rogercresseyi exhibit a number of behavioural responses to specific compounds (kariomones) present in salmon mucus, including isophorone and 6-methyl-5-hepten-2-one. These compounds assist in host location ‘off-host’ as well as identification and attachment processes ‘on-host’. In addition to mucus, the chemical composition of the skin and flesh of the salmon are also important in host selection. Both Atlantic and Pacific species of salmon exhibit this positive attraction to sea lice. However lice responses are inhibited or repelled if non-host compounds such as 4-methylquinazoline and 2-aminoacetophenone from non-salmonid, fish are detected. Kariomones are host-derived chemical cues which induce a behavioural or physiological change in a member of another species to the benefit to the receiver. These kariomones may be masked by a range of vegetable based compounds. Lab-based in vitro assessments have identified a library of potential replacement ingredients and some of these have been assessed in fish challenge studies.

Work plan 2012

- Recruit post-doctoral scientist to WP2
- In vitro assessment: Y-tube rheotaxis, frontal filament model
- In vivo initial assessment of masking compounds in feed
- In vivo assessment of immune compounds in feeds

Recruitment

A postdoc/researcher has successfully been recruited as principle scientist to the work package.

In vitro assessment

A range of in vitro systems (Y-tube rheotaxis, frontal filament model) have been used to assess the effects of salmon-host molecules on the behavioural responses of sea lice (both L. salmonis and C. rogercresseyi). Significant responses have been observed towards these salmon-host molecules for both species.

Masking compounds have been assessed in conjunction with the host molecules, used within these two in vitro systems. Significant reductions in positive rheotaxis (directional response) of up to 90% were observed in both lice species with the use of the masking compounds. In addition there was a significant reduction in the numbers of C. rogercresseyi deploying their frontal filament when exposed to host molecules combined with masking compounds. Further work is needed to develop an effective frontal filament assay against L. salmonis.
**In vivo assessment of masking compounds**

Initial *in vivo* challenge studies have also been conducted for both *L. salmonis* and *C. rogercresseyi*. Positive effects of isothiocyanate against both *L. salmonis* and *C. rogercresseyi* were observed. However, the pure compound was very volatile, resulting in large losses during feed production and storage. A more stable form of isothiocyanate is present as its precursor glucosinolate in many plant ingredients. Here isothiocyanate is liberated by enzymatic action during digestion. Specific plant compounds have been included in feed and assessed under *in vivo* challenge. Effect in *C. rogercresseyi* ranged between 28% and 9%. Recent results have also shown a 28% reduction in *L. salmonis* attachment (Figure 1). Further work will be conducted to dose as well as efficacy in combination with immune stimulants.

![Figure 2.1: The reduction in attached lice at 24 days post challenge following feeding with a plant based product (P). There was a 28% reduction (p<0.05) in lice levels compared to controls (C).](image)

An additional feeding study was performed to assess bioavailability of isothiocyanates. This time-course study was designed to investigate the modes and sites of absorption of isothiocyanates, their biotransformation and accumulation in target organs (including skin) by GC-MS. In addition to acting as repellents, these plant based compounds may exert biological effects in target tissues that could aid in the anti-parasitic protection. Assays are currently being conducted and will be reported during Q3-13.

The profile of host molecules varies over the life cycle. In many species sexual maturity increases the expression of compounds that are attractive to the opposite sex. Compounds that are anti-parasitic (such as 2-aminoacetophenone) may be preferentially expressed at this time, showing potential mates that the individual is more resistant to parasites. In a recent study oestrogen and testosterone fed fish showed significant differences to subsequent lice challenge (>45% reduction). Further work is required to assess the host specific compounds between the groups and identify any differences in expression. Microarray and qPCR highlighted a number of immune and physiological pathways that correlated to improved protection. This approach appears a useful research tool for both anti-attachment and immune work.

**In vivo assessment of immune compounds**

When the juvenile stages of sea lice are attached to salmon they are exposed to a potentially fatal immune response. To protect themselves they release a series of secretary/excretory products (SEP) into the host tissue, via salivary glands. Prostaglandins (PGE$_2$), alkaline phosphatase and a range of trypsin-like proteases have been identified as sea lice SEP’s. These factors have a significant immune-modulatory effect on a range of responses in Atlantic salmon including reduced respiratory burst, lower macrophage activity, increased apoptosis, necrosis, decreased numbers of mucosal cells and down-regulation of immune genes such as interleukin IL-18 and MHC-1. Immune suppression occurs at localised attachment sites, although a more generalised effect may occur with higher levels of sea lice infection. It appears that the release of the SEP’s may be triggered by the detection of the host molecules. There is a close association between attachment and subsequent immune modulation by the parasite.

Recent trials have shown that in fish fed a control diet, sea lice were able to successfully down regulate over 550 genes, many responsible for immune control. In the diet fed a combination of immune stimulants and nucleotides there was no down regulation of these genes. This correlated to improved protection of the diets from sea lice (50% reduction) and an effective immune response.

![Figure 2.2: The reduction in attached lice at 28 days post challenge following feeding with an immune stimulant (diets A & B). There was a 50% reduction (p<0.05) in lice levels compared to controls.](image)
Interleukin IL-1β at 5 days post challenge, 28 days post feeding

![Graph showing IL-1β expression]

Figure 2.3: The expression of interleukin 1 at 5 days post challenge, 28 days post feeding. Diet B showed a significant increase in expression of this gene compared to the other diets as well as non-infected controls. This profile correlated to the efficacy observed post-challenge.

Work Package 3: Immunomodulation of the host

WP3 addresses Immunomodulation of the host. The concept is that the sea lice release 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 interventions that can alleviate the down-playing effect of the secretory products, the idea has been to elucidate the underlying mechanisms of inflammation and anti-inflammatory processes.

The reporting of activities for 2012 is according to planned activities.

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

The Atlantic salmon macrophage cell line was used to study the modulatory effect of PGE2 in vitro (Fig. 3.1). Cultured TO cells were pretreated with PGE2 at 1 and 10 μM 30 min prior to stimulation with LPS (50 μg/well). Each well contains around 105 cells. There is a modulatory effect of PGE2 treatment on IL1β in result in delayed induction of expression while the effect on TNFα expression is not so obvious. The response for TNFα has in general been found to be variable.

We also included studies of chemokines that are known to play an important role for recruitment of inflammatory cells to the site of infection/tissue damage. Here (Fig. 3.2) we found that PGE2 significantly down-regulates the mRNA expression of CXCL-10 and CCL4 (MIPβ) as shown below. The effect of PGE2 treatment is particularly evident at 6h post LPS stimulation, seen for both CXCL10 and CCL4.

![Graph showing CXCL-10 and CCL4 expression]

These findings are clearly indicative of PGE2 playing a role in modulating inflammatory responses in vitro and the implications for these findings will be further studied in vivo.

Establish a method to identify proteins/components involved in host-parasite interaction, using a combined genomic/proteomic approach

The initial approach was to characterize the receptor for PGE-2 in salmon tissues. In brief the approach was to obtain the sequence of EP4 which has been shown to be important for immunomodulatory (dampening) effects following PGE-2 engagement. This was done through homology cloning and after obtaining the sequence the sequence was compared to existing sequences of other fish species as published. The results of the comparison are shown below:
In brief this shows that salmon EP4 is closely related to the zebrafish EP4a and b and less to chicken and further diversified from higher vertebrates EP4.

The next step was to elucidate the expression of EP4 in different tissue (organs). This was done by assessing the expression level at mRNA level (Fig. 3.4). There is differential expression in different tissues with spleen, gills, headkidney and skin showing high expression levels. Brain shows no expression.

The sequencing data has also showed that there is some variation at genome level for the EP4 receptor. There are two main findings. Firstly there is a mutation in the UTR region which can be found in all organs except for skin. This mutation is again accompanied by a silent mutation at position 668 (from G-C). In muscle samples there is a mutation at position 448 (T-C) changing the amino acid from leucine to proline. The importance of this mutation is not understood or known and we have taken an approach to model the potential impact of the 3-D structure of the molecule from this mutation. These studies are on-going.

In vivo fish (Atlantic salmon) challenge with copepodid

Atlantic salmon were challenged with copepods at standard doses (20 copepods per fish). Examination and sampling was carried out at approx. 4.5 weeks post challenge (challimus stage) and the infection rate was estimated.

Samples of skin were collected together with headkidney and spleen samples. Parallel samples were taken for histology and RNA-preservation. Fish were grouped by families and contrasted with regard to previously recorded susceptibility to sea lice infection, denoted highly resistant (HR) and low resistant (LR).

The figures show gene expression in skin for PCNA and GATA3 and spleen for PCNA infected low resistance (LR) and high resistance (HR) salmon. Skin samples were collected behind the dorsal fin. Non-selected and non-infected salmon were used as controls (the zero baseline); n=8 for each group. Data are presented as –ddCt values; p-values next to axis show differences between selected fish and controls. As can be seen from the preliminary data PCNA which is an indicator of cellular proliferation was expressed lower compared to controls in LR while compared to the HR group, PCNA was significantly lower expressed in the skin. The picture was different in spleen with PCNA more down-regulated in HR compared to LR groups. The GATA3 trends are not easily understood.

When in situ staining for PCNA is performed it is not evident that LR groups have lower number of positive cells than the HR group while preliminary findings indicate that infected fish have thicker epidermis and also more Goblet cells in the epithelial lining. These examinations are ongoing and will be reported in detail in next year’s report.

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

The salmon louse was described for more than 170 years ago in 1838 and in this respect a well-established species and it is today recognized as an economically highly important parasite. In spite of this, most aspects of the salmon louse biology are still poorly understood and detailed studies addressing key processes like molting, reproduction and host interaction are limited. Basic knowledge on key processes in the salmon louse will be the fundament to identify new targets for future sea lice control. One of the targets in WP4 is to elevate the basic knowledge about sea lice biology at some key points in the life cycle (e.g. copepodids, reproducing females) and to study the main biological processes (sensory system, host interaction, molting, nutritional handling and reproduction) in L. salmonis. The available salmon louse genome sequence is a key resource for the research activity and creates a simplified and time-saving starting point in all the studies. Selection of candidate
genes in our RNAi screening together with WP6 is facilitated by the genome sequence and we pick candidates both based on knowledge from other species or other criteria.

The research activities in WP4 were initiated during 2012 as the personnel were recruited. The group works in functional laboratories at Department of Biology and Department of Molecular Biology in close vicinity in the same building with access to complete instrument parks at both departments. During 2012 two post docs and three PhD candidates have been part of WP4. All PhD candidates have been enrolled in the PhD programs at Department of Biology or Department of Molecular Biology.

WP 4 is for simplicity divided into 3 parts:
1) Copepodid biology
2) Reproduction, germ cells differentiation and maturation
3) Endo and exocrine system in salmon louse

Work plan 2012
• Establish the research group of senior scientists
• Establish tools
  - Isolate full length genes, in situ probes, qPCR primers and production of proteins.
  - Establish assays for nuclear receptors
  - Design peptide antibody against candidate protein
  - Develop RNAi as a method used at the copepodite stage.
• Bioinformatics: Identify candidate genes, analyse homologs, determine gene repertoire.
• Perform in situ analysis in relevant lice stages.
• RNAi studies.
• Monitor gene expression, RT-PCR

1. Copepodid biology

Characterize the free-living to parasite transition in the salmon louse
The most critical step in the salmon louse life cycle is to infect a new and suitable host where the parasite can grow and reproduce. The first step in the process of infecting a new host is for the parasite to position itself optimal to encounter hosts at a reasonable distance. *L. salmonis* can only successfully infect salmonids but how it recognises a suitable host is unknown. The exact mechanism for host recognition is one the topics we seek answer to. Furthermore, once a suitable host has been recognised by the parasite it is imperative for the parasite to start feeding. To facilitate this, the salmon louse is dependent on controlling the host immune system which is done by releasing a set of components influencing the host immune ability. The free-living copepods are surviving on maternal provided energy and after infection of a suitable host they start feeding to gain energy to be able to molt into chalimus 1. This means that a large number of biological processes are initiated at this stage and one way to characterize these is through comparative transcriptomics like microarray assessment.

Chemosensory system in *L. salmonis*
All organisms are able to detect small molecules in the environment that give them information on food, predators, pathogens, and mates. Odour detection ability is essential for the survival of most animals, therefore both vertebrates and invertebrates have developed complex repertoires of chemosensory receptors to detect and distinguish chemical cues. These molecules are important in host recognition for parasites. In Arthropods, the majority of stimuli are recognized by members of multigene families, including Odorant-Binding Proteins (OBPs) and Chemosensory Proteins (CSPs), both involved in peripheral olfactory processing and two evolutionarily related chemosensory receptor families: Olfactory Receptor (OR) and Gustatory Receptor (GR), involved in smell and taste perception, respectively. Recently, a new subfamily of Ionotropic Glutamate Receptors (iGluR) has been identified, (i.e. Ionotropic Receptors (iRs)) and classified as a new family involved in olfactory perception (see Figure 4.2). Both iGluR and IR share characteristic ligand-binding domains (LBD), with three highly conserved residues in iGluRs responsible for direct glutamate binding in synapses. The iRs, localized in ciliated endings of the sensory neurons, have considerably more variable LBDs, and lack one or more conserved residues, changing their ligand specificity (see Figure 4.2 C).
Using bioinformatics methods, genes from different members of the chemosensory families, involved in smell and taste, were detected in salmon louse draft genome sequence. Three putative CSP, no OR or GR with confidence, and no OBP were detected. This is a surprising result and points towards a special sensory system in the salmon louse. During the search fragments from at least 36 putative members of the iGluR/iR family were detected, and further experimentally tested. The expression of iGluR/iR genes were analysed by PCR and full gene sequences were recovered using RACE approach. A total of 30 candidate genes were tested and 25 could be confirmed. The complete coding sequences were obtained and validated for 22 genes. Based on protein alignment of LBD, combined with phylogenetic analysis, members of the iGluR/iR family in salmon louse segregate into 11 iGluRs and 11 iRs. Observed diversity in conserved residues of iRs suggest broad ligand repertoire, which is planned to be tested in 2013. All 22 iGluR/iR genes were tested for tissue specific expression and three of them revealed antenna specific localization. Experiment was performed with regular PCR approach and the result will be validated with Q-PCR in 2013.

Orthologs of the IR co-receptors were found and in Arthropod species the co-receptors are known to have modulatory role in the signal detection. Using phylogenetic analysis one IR25a was identified and two salmon louse genes have highest similarity to the IR8a in other Arthropods. Expression levels of the two co-receptor genes, IR25a and one of the IR8a candidates (IR8a-1), were verified by Q-PCR in copepods and adult females. Both genes show relatively low expression levels. IR25a and IR8a-1 were further studied by RNAi experiments, aimed at altering host recognition. Although down regulation was detected (more significant in the case of IR8a) no visible phenotypic or behavioural changes were observed in the present set-up. More specific experiments involving RNAi will be conducted in 2013.

Gene regulation in parasitic copepods – a massive transition

Expression profiling using a 44k L. salmonis microarray (representing 12049 different genes) from settlement and during the next six days revealed 7586 (63%) differently regulated genes (Figure 4.3). The regulated genes could be classified into many distinct transcription profiles (Figure 4.3, all clusters except cluster 2 representing the unregulated genes).

The settlement of copepods on the host marks the beginning of the parasitic lifestyle for sea lice and is accompanied by initiation of feeding, growth and development, exposure to the host immune system and at the end molting into the chalimus stage. All these events are reflected in the detected changes in gene expression. By comparing the functions of differentially clustered genes certain patterns of enrichment is evident. One example is the genes that are up regulated prior to molting at day 6 which is enriched for functions in cuticle, metabolism and sterol homeostasis. Another example is cluster 15 (representing genes up regulated directly after settlement) contains genes typically linked to gluconeogenesis, lipid homeostasis and amino acid metabolism. In this study the ecdysteroid receptor (EcR) was highly expressed (Figure 4.4) at settlement, lowest at day 3 and up regulated again at day 6 which fits well to observations in arthropods in general. As shown in Figure 4.3, many of the salmon louse genes are unknown (green), but by fitting these into an expression profile, possible functions could be indicated.
Measurements for mRNA levels were performed for EcR and some EcR related genes (i.e. E74, E63e, E75, HR38, FTZ-F1, HR46) in the copepodid time series and in some other developmental stages (Figure 4.4). In all measured stages, the EcR is upregulated directly before molting as observed in the copepods. Two cuticle related genes (one structural protein and one carboxypeptidase) are upregulated before each molt, but only once during chalimus stage 1+2 and 3+4 respectively.

Figure 4.4: Expression profile of EcR in a time series of different developmental stages of the salmon louse (upper panel) and occurrence of the different developmental stages on the fish (lower panel). The time span where molting occurred, is highlighted with colours. Cop = copepodids, chal = chalimus, preA = preadult.

2. Reproduction, germ cells differentiation and maturation

Nuclear receptors

Several vital processes such as molting, germ cell maturation and sexual differentiation, and general growth are highly influenced by gene regulation. Nuclear receptors are important gene regulators that may be activated by specific chemical signaling molecules (ligands). The hypothesis is that molting and other maturation processes may be manipulated by carefully designed inhibitors or activators at low concentrations to block or disturb the timing of a process.

The activities in 2012 have included molecular characterization of Ecdysone receptor (EcR), its co-partner Ultraspiracle (USP) and alternative partner of USP named hormone receptor 38 (HR38). The work has revealed that EcR mRNA is alternatively spliced with several different starting points and different promoters. Peptide antibodies has been raised against EcR and shown to work in histology sections (more on EcR further below). HR38 has a female specific splice variant where coding for activation domain 1 is missing. Furthermore the ecdysone regulated protein 75 (E75) and its co-partner hormone receptor 3 (HR3) and hormone receptor 4 (HR4) have been characterized. Purified recombinant E75 protein appears to have a heme-group in the ligand binding pocket that indicate the use of NO (or CO) as signaling molecule. A cell culture based assay system for monitoring ligand activation and co-partner interaction has been designed and will be used for testing a range of possible ligands in 2013. Further studies of some of these receptors are dealt with separately.

Germline formation: role of nuclear receptor in germ cell development

Germ cells are formed early during embryonic development of an organism. It differs from somatic cells during embryogenesis as well as in later development. When germ cells are specified they are called primordial germ cells. They later migrate to gonads and differentiate into gametes. In addition to germline specification the germ cells attain a sexual identity before gametogenesis. To identify germ cell sexual identity, sex specific markers have been used. Furthermore sexual identity can also be deduced by studying expression of meiosis specific markers during development of an organism. Several of these processes are believed to be regulated or at least influenced by nuclear receptors.

a) Gene cloning and sequence analysis

Several nuclear receptors and germ cell marker genes have been identified. Two examples are HR4 nuclear receptor and Nanos germ cell marker that was identified by the use of bioinformatics tools and subsequently cloned and characterized as complete transcripts. Two Nanos genes were found Nanos1 and Nanos2. Interestingly Nanos1 have splice variants also. The sequence analysis predicts that Nanos has a zinc finger binding domain.

b) Gene expression, RT-PCR and qPCR

Salmon lice have 8 stages in their life cycle and each stage has a different pattern of gene expression. Extracted RNAs from developmental stages were used to prepare cDNA, RT-PCR and qPCR for gene expression analysis. The nuclear receptors shows variable pattern of gene expression in different stages as do the germ cell marker Nanos1 and Nanos2 (see Figure 4.5). The preliminary study shows that Nanos1 is expressed at all stages and with increased expression in earlier stages like in nauplius and copepodid stages, whereas Nanos2 shows almost only expression in later stage like adults stage and mainly in female and ovaries.
c) Whole-mount in situ hybridization

In situ hybridization is used for localization of gene expression in different tissues in an animal. A protocol for whole-mount in situ hybridization is under development with the goal to identify the germ cells in salmon louse and follow their development.

d) RNAi screening of different genes

RNAi is an efficient reverse genetic method to study the function of a gene. This method has been used to study the function of genes like E75-1, E75-2, HR3, Nanos1 and Nanos2 in salmon louse. Some of the genes like E75 induced high lethality after RNAi injections, while Nanos1 and 2 knock-down (about 40% mRNA reduction) showed relatively mild morphological changes in some of the animals (Figure 4.6). HR4 knockdown had a lethal phenotype and will have to be repeated.

Oocyte maturation

The mature eggs are enriched with nutrients for larva development and proper growth until nutrients are available from external sources. The major components of eggs are yolk proteins, lipids and carbohydrates. Lipoprotein receptor family plays vital role for incorporation of these nutrient components into maturing oocytes.

Two members from this lipoprotein receptor family (Vitellogenin receptor and Lipophorin receptor) are under characterization. Putative receptor genes have been identified in sea lice genome database. Two vitellogenin receptor genes (sLVitR1 and sLVitR2) are identified as potential candidates for further studies. The most promising gene based on sequence similarity (sLVitR1) has two isoforms where one is expressed in all development stages, including both male and female adult animals, while an unusual splice form (GC-AG intron) is specific to adult females. The relevance of the female specific splice variant is currently under investigation. The mRNA localization studies and gene knock down for sLVitR1 has been carried out in adult sea lice. The expression was located to ovaries, but not in maturing egg strings. Knock down experiments gave reduced fertility, but not as strong as expected if this is the vitellogenin receptor. The second putative vitellogenin receptor (sLVitR2) has an expected female specific expression profile and will be further studied in 2013. Similarly, also one gene of Lipophorin receptor (sLLpR) is recognized as a possible candidate for future work. Protein sequence comparisons of sLVitR1, sLVitR2 and sLLpR show high similar regions to other organisms including vertebrates, insects and crustaceans.

3. Endo and exocrine system in salmon louse

Molting; biology and regulation by hormone receptors in salmon lice.

The first part has been to study hormonal regulation of molting in the salmon louse. This has been achieved using RNAi. A distinct phenotype could be observed when EcR, postulated to be important in reproduction, where knocked down (Figure 4.8). Gene knock down was confirmed at the RNA level using quantitative PCR. Further on, EcR has also been localized in different stages of the louse. This was achieved using in situ hybridization to localize the mRNA. Moreover, studies regarding stage specificity of EcR were performed. Preliminary results show that EcR is more highly expressed at some stages compared to others. In silico studies of phylogenetic relationship between the salmon louse EcR and homologous genes from other species was also performed. Preliminary results are to be verified and expected published in 2013.
Exocrine factors in salmon Lice
Salmon lice are capable of sitting on and feeding from the salmonoid host for extended periods of time (more than a year) without giving rise to an immune response and forceful immune response from the fish. To accomplish this, the lice must somehow be able to manipulate the immune system of the fish. In this project we try to find what factors are important for this interaction and specifically we study the factors that the lice produce and excrete to the fish. We have localized a number of such glands. The next step in this project will be to identify gene products that are characteristic for these glands and determine their effect on the fish.

Gene expression profiles
In a knock-down study of USP, one of the partners of the ecdysteroid hormone receptor, strong consequences for the offspring was observed as almost no normal larvae were produced. Analysis of the gene-expression profile in knock-down adult female lice vs. control lice showed direct and indirect disturbance in the gene expression of many hundred genes. Many genes involved in early development, the main egg-yolk proteins (vitellogenins), regulators of vitellogenin synthesis, a large number of putative cuticle protein genes, transcripts of genes involved in metabolism as well as many transcripts for ribosomal proteins were strongly affected. The results from this study are submitted to publication.

Endocrine factors in salmon lice
Endocrine factors regulating homeostasis, development, digestion and reproduction are virtually unexplored in lice. In 2012 we have initiated searches for endocrine factors using a bioinformatics approach to identify target genes (peptide hormones) or genes involved in the synthesis of hormones. We have identified promising candidates and are currently in the initial phase of testing these.

The target of rapamycin (TOR) pathway
The TOR pathway is essential in a number of cellular processes. In this project we want to specifically focus on the involvement of this pathway in nutrient signaling and subsequent egg production in adult females. This project is delayed due to the hiring of a PostDoc on maternity leave. Despite this a number of genes have been tested in RNAi screens with significant results. Further evaluation of the results will commence in 2013.

Work Package 5: LiceBase

Introduction – The Salmon Louse Genome
The availability of the complete genome sequences of many model organisms has provided life-science researchers with a previously unseen wealth of information. Recent advances in sequencing technology (so called 2nd generation sequencing) have made genome sequencing available to an ever-larger research community, including also non-classical model organism genomes, such as the salmon louse (*L. salmonis*), which has been sequenced by the Salmon Louse Genome Project (sealouse.imr.no) or the water flea (*Daphnia pulex*).

The raw nucleotide sequence is however not very useful by itself. It needs to be augmented by a good annotation of potential functional regions, the genes, encoded in the genome. Together with the Ensemble group at the EMBL-EBI we are currently annotating the sea louse genome. The genome and annotation will be made available through LiceBase and be improved iteratively in a so-called curation process, when new data on genes become available.

High-throughput sequencing of mRNA (RNA-seq) of different developmental stages, discovery of genomic variation (e.g. SNPs), and large-scale phenotype screens of gene-function (e.g. via RNAi induced gene knock-down) are among the standard tools of functional genomics. The application of all the functional genomics tools developed for the salmon louse is critically dependent on searching the genome in order to improve the annotation and aiding the process of curating the genome and its annotation. Being able to efficiently mine the genome for genes, pathways and regulatory networks is key to designing good follow up experiments.

Developing LiceBase as an Integrative Model Organism Database
Functional genomics experiments performed in LiceLab, and elsewhere, generate a wealth of data that needs to be linked to the genome and made accessible for researchers inside the Sea Lice Research Centre as well as to the general public for dissemination of scientific results. Efficient search and data mining tools are needed to benefit from the data. LiceBase has been intended to become the primary resource for genomic data directed towards the whole sea lice research community. To achieve this goal, we have set out to
conduct a comprehensive requirements analysis by studying the user community and the available data sources. To be able to provide a large set of functionality with the available resources for software development, we decided to adhere to open standards and re-use existing software wherever possible. We also set a focus on open-source solutions for reasons of flexibility and extensibility.

After a concise evaluation of existing software to solve the tasks of management, mining, and integration of genomics data, we have reached the conclusion that the GMOD (gmod.org) set of tools provides the most adequate and complete collection of open-source software to implement LiceBase. GMOD tools have already been applied to build model organism databases such as FlyBase (flybase.org), WormBase (wormbase.org), wFleaBase (wfeabase.org), and many others (gmod.org/wiki/MOD). Another argument for the use of GMOD tools is its active and responsive user-community. We have focused initially on establishing contact with the developer community via visiting the GMOD meeting 2012 in Washington, USA.

LiceBase – Architecture and Implementation
LiceBase is being built using core GMOD components augmented with in-house software and few components developed as our own code-base inside the project (see Figure 1).

The system uses CHADO (gmod.org/wiki/Chado) and PostgreSQL (postgresql.org) as its back-end to store all genome-related information. CHADO is an extensible database-model aimed at supporting a large range of genomic regions, and also storage of functional genomics data and ontologies. At a later stage this setup will enable to manage several replicated instances of the genome annotation for increasing performance and security.

On top of this back-end, we have initially planned to provide two web-applications:

A genome-browser based on GBrowse (gmod.org/wiki/GBrowse), and a system providing access to web-pages for organisms, genotype-phenotype annotations and storage of experiments based on Tripal (tripal.info).

GBrowse provides visualization of the genome and data through tracks. These tracks together represent information from multiple data sources in a linear representation on top of the raw genome sequence. Tracks can relate to any genomic feature, e.g. genes and transcripts, or quantitative data like GC-content or RNA-seq coverage. The user interface is highly customizable, supports multiple genomes, and users can also upload their own custom tracks.

Tripal is based on the Drupal content management system, and provides dynamic web content generation for the genome annotation to present internally to the Centre and also to the general public. It enables creation of custom web pages for organisms, phenotypes, genome annotation features, and ontologies.

From its roots as a content management system, Tripal/Drupal also supports many Web 2.0 features, like blogs and forums as well as site-wide searches. In this project we have extended Tripal with functionality for managing and searching RNAi experiments.

We are also aiming at providing a unified user authentication mechanism with single sign-on (only one entry of username-password) for all applications allowing for improved usability and at the same time a high level of data security. For this purpose we are using an authentication mechanism based on the Security Assertion Markup Language (SAML) which is an open web-standard for secure federated authentication and authorization services. Within this project we have written software plugins that integrate the genome browser with this solution.

In the course of 2012 we have set-up and integrated the software solutions on two computer systems (See Figure 2 for screen-shots). We also have installed a high-performance database server to host the genome annotation databases. We are planning to enter a beta-testing phase in early 2013 and a final release of the production system jointly with the release of the genome annotation.

Our system development benefits from access to open-source software projects and in line with this philosophy, we contribute the code and documentation developed in context of LiceBase back into the respective open-source projects. So far we have contributed our authentication module to GBrowse2 (https://github.com/mdondrup/gBrowse) and additional documentation to Tripal (tripal.info/documentation/tripal_saml_integration).

In this way we contribute to the further success of the open-source projects and also gain higher visibility in the computational life science community.

LiceBase is developed in collaboration with the ELIXIR.NO project – a nationally funded project aimed to build a Norwegian node of the pan-European bioinformatics infrastructure ELIXIR (an ESFRI project). The proposal for a Norwegian ELIXIR Node, submitted in 2012, includes LiceBase as one of the marine genomics resources to be offered by the Norwegian node. The collaboration between SLRC and ELIXIR.NO is beneficial for both parties – SLRC benefitting from infrastructure investments and resources performed within ELIXIR.NO and LiceBase serving as a pilot marine genomics resource within ELIXIR.NO.

Ontologies for Sea Lice Research
Ontologies play an important role in biomedical research. They provide a way to represent knowledge in a standardized and structured way that supports automatic interpretation and search. Probably the most prominent example for a bio-medical ontology is the Gene Ontology (GO, geneontology.org), that will be used to annotate the lice genes. For the annotation of experiments on sea lice, ontologies are already playing an important role for the annotation of developmental stages within LiceBase.
In 2012, we have established the Sea Lice Ontology Working-Group (SLOW) with the aim of developing ontologies at the Sea Lice Research Centre. Ontology development needs to be done jointly with the domain experts in order to integrate them tightly with the formalization process. That way, we have finished developing an ontology of sea lice development. The Sea Lice Development Ontology (see Figure 3) has been imported into LiceBase, and can be used for annotation of RNAi experiments. Furthermore, we have initiated the development of an anatomical atlas ontology of the salmon louse, which will be completed during 2013 after more images of sea lice body parts from different developmental stages are available.

**Figure 5.1:** Sketch of the architecture of LiceBase and its components.

**Figure 5.2:** The organism page of *L. salmonis* with links to annotation resources on the right. (A) The RNAi experiment with a list of experiments filtered for the phenotype “Female reproduction” and thumbnail images of the results in the left column (B). A preliminary assembly of the *L. salmonis* genome in the GBrowse2 genome browser. Two different gene predictions are visible as “Augustus” and “EBI” are depicted for comparison (B).
Work Package 6: LiceLab

The LiceLab 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.

Given the large number of RNAi screens (100/year) to be performed at SLRC, we have modified a number of procedures in the set-up of the experiment and the logistics during the experiment and during the evaluation of the screens. Major activities have included:

- Development of a systematic and predictable identification of all samples
- Training of personnel to be able to assist in set up RNAi screens. 11 persons are currently trained to handle lice during the procedures. All PhD students and Postdocs at SLRC in Bergen have either obtained or are in the process of obtaining certificate to handle experimental animals.
- During set-up of RNAi screen, the lice are exposed to handling and this result in unspecific mortality. Unspecific mortality leads to increase need for animals and results that are harder to interpret. Procedures for set-up have been developed during 2012 to significantly reduce handling of the animals and at the same time decrease time consumption.
- Developed method for increased sensitivity in assessment of gene knock down.

RNAi screening project

During 2012, 51 RNAi screen experiments were performed at the wet-lab facilities at UiB and IMR. Evaluation of RNAi screens from 2012 is on-going and results on specific genes will be reported from WP4. Table 6.1 present an overview of all experiment and results obtained so far.

<table>
<thead>
<tr>
<th>total RNAi screen</th>
<th>total gene targets</th>
<th>knock-down tested by QPCR</th>
<th>visible phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>51</td>
<td>45</td>
<td>30</td>
<td>12</td>
</tr>
</tbody>
</table>

Table 6.1

Total RNAi screens reflect the total number of fragments injected in lice (each fragment is injected into 30 lice and distributed on 3 fish). Total gene targets: this number shows how many different genes have been tested, i.e. total numbers minus controls and gene that have been run again to accommodate for genespecific sampling. RNAi knock down experiments have to be confirmed by quantitation of the target gene using a technique called QPCR. Phenotypes are scored on a morphological basis inspecting whole animals and sections of these: Typical phenotypes included lack of development, termination of reproduction and mortality (recorded throughout the experiment).

RNAi screening on planktonic stages

Initial trials using previously described methods for RNAi silencing in planktonic stages of salmon lice (Campel et al. 2009) were not successful. An alternative procedure was developed and the results show that it is possible to knock down genes in nauplii and copepodids (6 target genes tested). These stages are readily available in high numbers and can be dealt with in simple experimental systems. The results obtained give promise for the development of high throughput RNAi screening procedures; work on this will be continued in 2013.

Production of other material and experiments

- 6 strains of lice (Ls1a, Lsr, LsGulen, LsAustevoll, LsBjugn and LsFrøya ) maintained.
- Lice material from various salmon louse laboratory strains has been delivered to Patogen, Novaris and NVH.
- Material for RNA purification sampled for academic partners in Bergen and Oslo.
- Testing heritability of RNAi induced gene knock-down. In addition infection success of offspring was assessed.
- Testing host change and mating capacity of males subjected to RNAi knock down.

Revision of the salmon louse life cycle and growth of larval stages

For half a century, the Lepeophtheirus life cycle has been regarded as the only copepod life cycle comprising ten instars as confirmed in four different species, including L. salmonis. Here we prove that the accepted life cycle of the salmon louse is wrong. By observations...
of chalimus larvae molting in incubators and morphometric cluster analysis, we show that there are only two chalimus stages: chalimus 1 (comprising the former chalimus I and II stages which are not separated by a molt) and chalimus 2 (the former chalimus III and IV stages which are not separated by a molt). Consequently the salmon louse life cycle has only eight instars, as in other genera of caligid sea lice and copepods in general. A significant instar growth among the chalimus larvae was observed, explaining the previous misconception on the number of chalimus stages in the life cycle. This work was performed in collaboration with the Institute of Marine Research in Bergen, The university of Stirling and The Natural History Museum in London. Two articles are ready for publication in the first half of 2013. Figure 1 show the chalimus 1 and chalimus 2 stage and the exuvia shed by the larvae upon molting.

Figure 6.1: Chalimus larvae and shed exuviae belonging to the chalimus 1 (A, B) and the chalimus 2 (C, D). The exuviae shown are the actual exuviae shed by the depicted larvae. TL= total length, CL= cephalothorax length, CW= cephalothorax width.

Wet lab construction and installation
In 2012 a new hatchery and new 1x1 m tanks have been installed at the Sea lice laboratory in Bergen. The work on providing sufficient water supply into the labs has been finalized and new single fish tanks have been evaluated. Construction of a new hatchery with increased capacity and new 32mm incubators started at IMR. A new sea lice laboratory has been established at EWOS Dirdal.

At LiceLab, UiB, Single fish tanks are primarily used to study the effects of RNAi, vaccines and drugs on mobile stages of sea lice. A new single fish tank design (Figure 1) was tested in 2012. The test show that the fish thrive and grow well and that the new tanks can host a wider size range of hosts. However, the need for several improvements was identified. A new design has been developed and a simple yet promising test has been carried out.

Figure 6.2: Single fish tank system

A new hatchery with re-designed wet tables and water supply was built at the SLRC lice lab at HFB early 2012. The new water supply provides 100 individual adjustable outlets with a capacity to supply 100 racks holding 16 small 32mm incubators each. Racks for 800 incubators and c 350 incubators have been made. The new hatchery and incubators works very well. In two neighboring rooms ten 500 liter fish tanks (1x1m²) have been installed (4+6 tanks). An alarm system for single fish tanks, shelf tanks and regular 1x1m tanks has been developed and emergency air supply has been installed.
INTERNATIONAL COOPERATION

The research partners in SLRC are world leading within their field of research and have an excellent international network. Four of five industrial partners are worldwide market-leaders and have a broad international activity. International cooperation has been developed in 2012 directly from the centre but also based on new and existing individual networks.

SLRC is partner in a project funded by the research council of the Faroe Island and aims at identifying hatching enzymes in the salmon louse. SLRC through WP4 and WP6 is involved in several aspects in this study and some of the experimental work will be carried out in Bergen.

LiceBase (WP5) is developed in collaboration with the ELIXIR.NO project – a nationally funded project aimed to build a Norwegian node of the pan-European bioinformatics infrastructure ELIXIR (an ESFRI project). The proposal for a Norwegian ELIXIR Node, submitted in 2012, includes LiceBase as one of the marine genomics resources to be offered by the Norwegian node. The collaboration between SLRC and ELIXIR.NO is beneficial for both parties – SLRC benefitting from infrastructure investments and resources performed within ELIXIR.NO and LiceBase serving as a pilot marine genomics resource within ELIXIR.NO.

UiB and LiceBase have established good contact with the GMOD project developers and users. The GMOD project is an international open-source project with a large number of contributing organizations. So far, LiceBase has profited from the project by using the software itself, and receiving valuable support via the GMOD community support (gmmod.org/wiki/GMOD_Community_Support) and taking part in the community meeting during planning and set-up of the software and implementation of new features. UiB has started contributing a small amount of documentation to Tripal and code for SAML based single sign-on authentication which is currently under review. The intention is to foster this kind of informal collaboration as it has great benefits for all parties involved.

EWOS Innovation is a funding partner in two international research projects connected to SLRC. A 3-year grant assisted project in Chile, CONYCT, has been implemented in Chile. A post-doctoral scientist Chris Hawes has been recruited. The project is assessing the early host parasite relationship between three salmon species (Oncorhynchus kisutch, Salmo salar and Oncorhynchus mykiss) to sea lice. Techniques include using antibody, qPCR and metabolomic approaches such as NMR. Anti-attachment and immune compounds in the diet will also be screened as part of the project. The group in Chile is collaborating with the University of Aberdeen UK, to optimise the molecular techniques used in the project. The second project is a 2-year ENGAGE grant, EG: Anti-attachment factors in feed and their effects on the host-parasite relationship: the salmon and sea lice model has been awarded in partnership with the Atlantic Veterinary College, Prince Edward Island. The programme will assess the effects of dietary modulation on sea lice attachment and immune responses.

SLRC researchers have been invited as speakers at international seminars in Chile, The Faroe Islands and UK. These seminars are important arenas for development of future collaboration.

RECRUITMENT

By the end of 2012, all planned PhDs and Postdoc have been engaged in the centre. The recruitment process was longer than expected, but it has been important to use sufficient time to ensure hiring of well qualified personnel at SLRC. Master and PhD students within the scientific areas for SLRC are attractive personnel for the authorities and industrial companies, but even if it has been challenging to recruit the required personnel, we find that the centre is an attractive area for career development. For future recruitment, we plan to use presentations at seminars and different media to attract new personnel in addition to the traditional channels. The newly recruited personnel in SLRC have a variation in background and skills, a requirement to cover all the various aspects of the scientific activity in the centre.

Five PhD students have been recruited during the first half of 2012, and their PhD workplan have been presented at the centre workshops. PhD projects started in 2012:

- Molting; biology and endocrine regulation (PhD cand. L. Sandlund)
- Reproduction; germ cell differentiation and maturation (PhD cand. P. Bhattachan)
- Immunomodulation of the host – local inflammatory responses (PhD cand. H. Holm)
- Salmon lice and chemotherapeutants: Efficacy, screening and administration (PhD cand. Stian M Aaen)

Master students have been recruited in 2012 at UiB which is the only partner where master students are educated. Some of the master theses will be in collaboration with both the academic and industrial partners. During parts of 2012 two master students from University of Autonomous University of Barcelona and University of Barcelona have been working at SLRC through the Erasmus programme.

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. The number of females in SLRC is satisfactory, but in the long term more female postdocs/researchers are expected to become principle investigators in new SLRC-projects. New recruitment is planned to be 50% females and 50% males. The table below shows the current personnel in SLRC, including board members:
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<thead>
<tr>
<th>Category</th>
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<th>%</th>
<th>No Male</th>
<th>%</th>
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<tr>
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<tr>
<td>Postdocs &amp; Researchers</td>
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<td>46.5%</td>
<td>23</td>
<td>53.5%</td>
<td>43</td>
</tr>
</tbody>
</table>

Not all the Principle investigators are working full time for SLRC.

COMMUNICATION AND DISSEMINATION ACTIVITIES

Visibility, flow of information and communication of the development are important parts of the activities in SLRC. To ensure a best possible practice, guidelines for communication, affiliation and acknowledgement were established by the beginning of the year. Templates for presentations and posters have been made to facilitate recognition of SLRC as a centre.

A public web site www.slrc.no is the main tool for distribution of information on the research activities in SLRC. The web site was launched in June, and will be extended and updated with the development of the centre. A closed website for SLRC-members only is established for management of internal information and activity.

The SLRC members have been represented at various seminars and conferences through 2012, but “Sea Lice 2012” was the main arena for presentation of SLRC research activities. Sea Lice 2012 was arranged in Bergen 21–23 May with the Institute of Marine Research as leader of the Organising Committee. In addition, Frank Nilsen and Tor Einar Horsberg from SLRC were members of the National Scientific Committee for Sea Lice 2012. The goal of the International Sea Lice Conference series is to bring together international research groups and regulatory agencies to foster collaboration and communication on sea lice research. SLRC were represented by three key-note speakers at the conference and three of eight sessions were chaired by SLRC WP-leaders. Several posters were presented by the partners.

The research activity in SLRC has been communicated through various channels, both by the academic and industrial partners in 2012:

- Scientific Publications: 6
- Presentations at meetings and seminars: 20
- Poster-presentations: 3
- Media/Press coverage: 5
- Presentations towards public in general: 4

Most of the presentations have been directed towards national and international scientific community and industrial companies. The centre and research activities have also been presented for different levels of the Norwegian authorities.
## ATTACHMENT TO THE REPORT

### KEY RESEARCHERS

<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
<th>Main Research Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frank Nilsen (20%)</td>
<td>UiB</td>
<td>WP1: Chemotherapeutants WP4: Molecular parasitology – the basis for novel treatment methods</td>
</tr>
<tr>
<td>Tor Erlar Honsberg (40%)</td>
<td>NVH</td>
<td>WP1: Chemotherapeutants</td>
</tr>
<tr>
<td>Simon Wadsworth (50%)</td>
<td>EWOS Innovation</td>
<td>WP2: Anti-attachment</td>
</tr>
<tr>
<td>Øystein Evensen (25%)</td>
<td>NVH</td>
<td>WP3: Immunomodulation of the host</td>
</tr>
<tr>
<td>Rune Male (20%)</td>
<td>UiB</td>
<td>WP4: Molecular parasitology – the basis for novel treatment methods</td>
</tr>
<tr>
<td>Sussie Dalvin (80%)</td>
<td>IMR</td>
<td>WP4: Molecular parasitology – the basis for novel treatment WP6: LiceLab</td>
</tr>
<tr>
<td>Inge Jonassen (10%)</td>
<td>UiB</td>
<td>WP5: LiceBase</td>
</tr>
<tr>
<td>Lars Hamre (50%)</td>
<td>UiB</td>
<td>WP6: LiceLab</td>
</tr>
</tbody>
</table>

### PROFESSOR II / VISITING RESEARCHERS

<table>
<thead>
<tr>
<th>Name</th>
<th>Affiliation</th>
<th>Nationality</th>
<th>Topic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kevin Glover (20%)</td>
<td>IMR</td>
<td>UK</td>
<td>WP3, WP6, WP4</td>
</tr>
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</table>

### POSTDOCTORAL RESEARCHERS WITH FINANCIAL SUPPORT FROM THE CENTRE BUDGET

<table>
<thead>
<tr>
<th>Name</th>
<th>Nationality</th>
<th>Period</th>
<th>Topic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Christiane Eichner</td>
<td>German</td>
<td>Sept 2011-May 2013</td>
<td>WP4: Gene regulation</td>
</tr>
<tr>
<td>Te-Chuan Guo</td>
<td>Taiwan, PRC</td>
<td>Sept 2011-Sept 2016</td>
<td>WP3: Immunomodulation of the host</td>
</tr>
<tr>
<td>Anna Komisarczuk</td>
<td>Polish</td>
<td>Feb 2012-Jan 2016</td>
<td>WP4: Olfactory receptors</td>
</tr>
<tr>
<td>Michael Dondrup</td>
<td>German</td>
<td>Feb 2012-Jan 2016</td>
<td>WP5: LiceBase</td>
</tr>
<tr>
<td>Kiranpreet Kaur</td>
<td>Indian</td>
<td>Mar 2012-Mar 2016</td>
<td>WP1, Chemotherapeutants</td>
</tr>
<tr>
<td>Amy Gamil</td>
<td>Sudanese</td>
<td>Apr 2012-Mar 2013</td>
<td>WP3, Immunomodulation of the host – cloning and characterization of the EP4 receptor</td>
</tr>
<tr>
<td>Stanko Skugor</td>
<td>Croatian</td>
<td>June 2012-Feb 2017</td>
<td>WP2: Anti-attachment</td>
</tr>
<tr>
<td>Ana-Cathrine Øvregård</td>
<td>Norwegian</td>
<td>May 2012-Oct 2014</td>
<td>WP4: Exocrine secretion and immunomodulation</td>
</tr>
<tr>
<td>Marit Bakke (50%)</td>
<td>Norwegian</td>
<td>June 1 2012-May 30 2015</td>
<td>WP1, Chemotherapeutants</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Name</th>
<th>Nationality</th>
<th>Period</th>
<th>Topic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Christiane Trösse</td>
<td>German</td>
<td>Sept 2011-Apr 2014</td>
<td>Connected to WP4</td>
</tr>
<tr>
<td>Kari OH Helgesen</td>
<td>Norwegian</td>
<td>Sept 2011-</td>
<td>Connected to WP1</td>
</tr>
</tbody>
</table>

### PHD STUDENTS WITH FINANCIAL SUPPORT FROM THE CENTRE BUDGET

<table>
<thead>
<tr>
<th>Name</th>
<th>Nationality</th>
<th>Period</th>
<th>Topic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liv Sandlund</td>
<td>Norwegian</td>
<td>Feb 2012-Jan 2016</td>
<td>WP4: Melting, biology and regulation</td>
</tr>
<tr>
<td>Punith Battachan</td>
<td>Nepali</td>
<td>Mar 2012-Feb 2016</td>
<td>WP4: Germline formation and maturation</td>
</tr>
<tr>
<td>Mohammad T. Kahn</td>
<td>Pakistani</td>
<td>Aug 2012-Aug 2016</td>
<td>WP4: Development and maturation of Oocytes</td>
</tr>
<tr>
<td>Stian March Aasen</td>
<td>Norwegian</td>
<td>Mar 2012-Feb 2015</td>
<td>WP1: Salmon lice and chemotherapeutants: Efficacy, screening and administration</td>
</tr>
<tr>
<td>Helle Holm</td>
<td>Norwegian</td>
<td>Sept 2012-Aug 2016</td>
<td>WP3: Immunomodulation of the host – local inflammatory responses</td>
</tr>
</tbody>
</table>

### MASTER DEGREES

<table>
<thead>
<tr>
<th>Name</th>
<th>Nationality</th>
<th>Period</th>
<th>Topic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ewa Harasimczuk</td>
<td>Norwegian</td>
<td>Aug 2012-Jul 2013</td>
<td>WP4/WP6</td>
</tr>
<tr>
<td>Inger Tølds</td>
<td>Norwegian</td>
<td>Aug 2012-Jul 2013</td>
<td>WP4</td>
</tr>
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</table>

### TECHNICIANS WITH FINANCIAL SUPPORT FROM THE CENTRE BUDGET

<table>
<thead>
<tr>
<th>Name</th>
<th>Nationality</th>
<th>Period</th>
<th>Topic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lars Are Hamre (50%)</td>
<td>Norwegian</td>
<td>Sep 2011-Aug 2016</td>
<td>WP6: LiceLab</td>
</tr>
<tr>
<td>Enrique Perez (50%)</td>
<td>Spanish</td>
<td>Mar 2012</td>
<td>WP6: LiceLab</td>
</tr>
<tr>
<td>Per Gunnar Espeland (50%)</td>
<td>Norwegian</td>
<td>Aug 2012-Dec 2012</td>
<td>WP6: LiceLab</td>
</tr>
<tr>
<td>Heidi Kongshaug (50%)</td>
<td>Norwegian</td>
<td>Jun 2012-Jun 2016</td>
<td>WP4 and WP6</td>
</tr>
<tr>
<td>Melanie Koenig (30%)</td>
<td>German</td>
<td></td>
<td>WP4, Immunomodulation of the host – cloning and characterization of the EP4 receptor</td>
</tr>
<tr>
<td>Svenn Grindhaug</td>
<td>Norwegian</td>
<td>Oct 2012-Dec 2012</td>
<td>WP5: LiceLab</td>
</tr>
</tbody>
</table>

### TECHNICIANS WORKING IN SLRC PROJECTS WITH FINANCIAL SUPPORT FROM OTHER SOURCES

<table>
<thead>
<tr>
<th>Name</th>
<th>Nationality</th>
<th>Period</th>
<th>Topic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lars Are Hamre (50%)</td>
<td>Norwegian</td>
<td>Sep 2011-Aug 2016</td>
<td>WP6: LiceLab</td>
</tr>
<tr>
<td>Wenche Telle (50%)</td>
<td>Norwegian</td>
<td>Jan 2012-Aug 2016</td>
<td>All projects in WP4</td>
</tr>
<tr>
<td>Rune Landsem</td>
<td>Norwegian</td>
<td>Sept 2011-</td>
<td>WP1, WP2 and WP3</td>
</tr>
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</table>
ADMINISTRATIVE PERSONNEL WITH FINANCIAL SUPPORT FROM THE CENTRE BUDGET

<table>
<thead>
<tr>
<th>Name</th>
<th>Nationality</th>
<th>Period</th>
<th>Topic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frank Nilsen</td>
<td>Norwegian</td>
<td>Sept 2011-Aug 2016</td>
<td>Centre Leader</td>
</tr>
<tr>
<td>Ingunn Wergeland</td>
<td>Norwegian</td>
<td>Jan 2012-Dec 2016</td>
<td>Centre Coordinator</td>
</tr>
</tbody>
</table>

STATEMENT OF ACCOUNTS 2012

All figures in 1000 NOK

**Funding**

<table>
<thead>
<tr>
<th>Source</th>
<th>Account</th>
<th>Budget</th>
</tr>
</thead>
<tbody>
<tr>
<td>The Research Council Norway</td>
<td>8 538</td>
<td>9 577</td>
</tr>
<tr>
<td>University of Bergen (Host institution)</td>
<td>3 716</td>
<td>3 544</td>
</tr>
<tr>
<td>Other public financing *</td>
<td>4 299</td>
<td>4 568</td>
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<tr>
<td>Enterprise partners *</td>
<td>8 524</td>
<td>6 700</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>25 097</td>
<td>24 389</td>
</tr>
</tbody>
</table>

**Costs**

<table>
<thead>
<tr>
<th>Source</th>
<th>Account</th>
<th>Budget</th>
</tr>
</thead>
<tbody>
<tr>
<td>University of Bergen (Host institution)</td>
<td>9 904</td>
<td>10 063</td>
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<tr>
<td>IMR **</td>
<td>2 375</td>
<td>3 450</td>
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<tr>
<td>Uni Research</td>
<td>234</td>
<td>500</td>
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<tr>
<td>NMH</td>
<td>3 889</td>
<td>6 381</td>
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<tr>
<td>Enterprise partners</td>
<td>6 605</td>
<td>3 995</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>25 097</td>
<td>24 389</td>
</tr>
</tbody>
</table>

* Group of partners

**In the financial reporting for 2012, partner IMR has not used the valid RCN rules for calculation of personnel costs & hourly rates. IMR has been asked to make a correction in 2013 for the numbers reported in 2012 or ask the SLRC Board for acceptance of the deviation.**

SLRC PUBLICATIONS 2012


The total activity for SLRC in 2012 was 25,097 mill NOK compared to a budget of 24,389 mill NOK. Unused funding from RCN and the industrial partners are transferred to 2013.