



Sea Lice Research Centre – SLRC update

Christiane Eichner
University of Bergen



SeaLiceResearchCenter (SLRC)



<http://www.slrc.no/>



What is an SFI?

SFI = Centre for Research based Innovation

- One of three “centre funding” from Norwegian Research Council (NRC)
- Purpose: build up and strengthen Norwegian research groups that work in close collaboration with partners from innovative industry and innovative public enterprises
- Selection based on “free competition” between diverse applications
- NRC provides 5+3 years of funding (~1.3 mill Euros/year), requires 50% own funding (> 25% industrial funding)



9 Partners in SLRC

Academic Partners:

- University of Bergen
- Norwegian School of Veterinary Science
- Institute of Marine Research
- UNI Research AS

Industrial Partners:

- Novartis Animal Health AG
- EWOS Innovation AS
- Patogen Analyse AS
- Marine Harvest ASA
- Lerøy Seafood Group ASA



Goal for SLRC

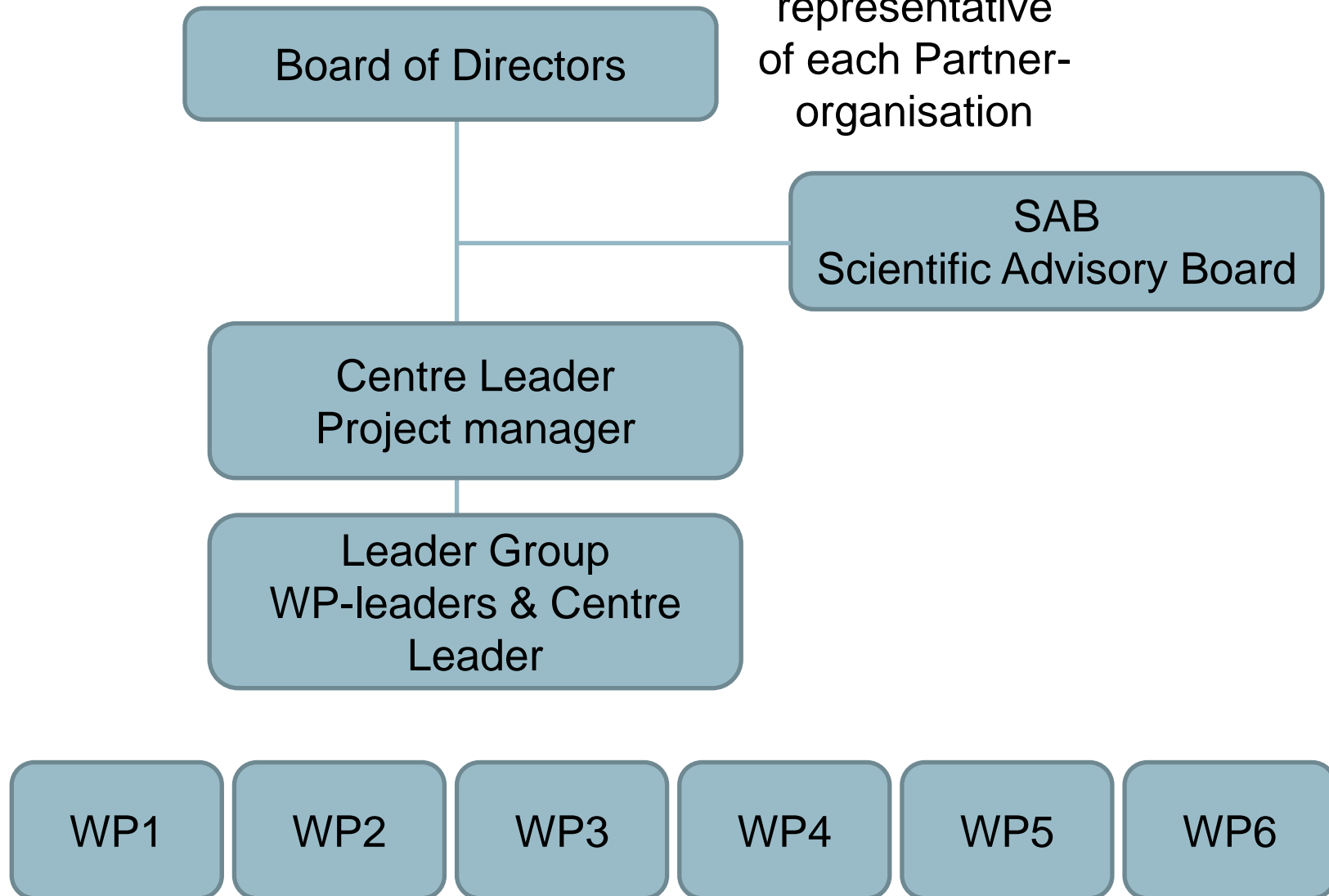
Research to develop new control measures against sea lice.

Main goal:

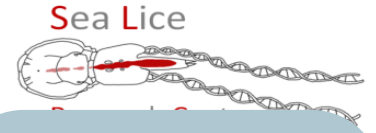
The Sea Lice Research Centre aims at becoming world leading on research on salmon louse and related parasites. The nature of the centre will facilitate development of new methods for lice control and **shorten the time from basic research to new products and tools for parasite control in the aquaculture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

Organisation

representative
of each Partner-
organisation



SLRC Leadership



Centre Director



Prof. Frank Nilsen

WP1
Medicine & Resistancy



Prof. Tor E.
Horsberg

WP2
Antiattachment



Dr. Simon
Wadsworth

WP3
Immunomodulation



Prof. Øystein
Evensen

WP4
Molecular parasitology
(new control methods)



Prof. Rune Male

WP5 LiceBase
(genome resources/
integrated database)



Prof. Inge Jonassen

WP 6 LiceLab
(wetlabs, experimental facility)

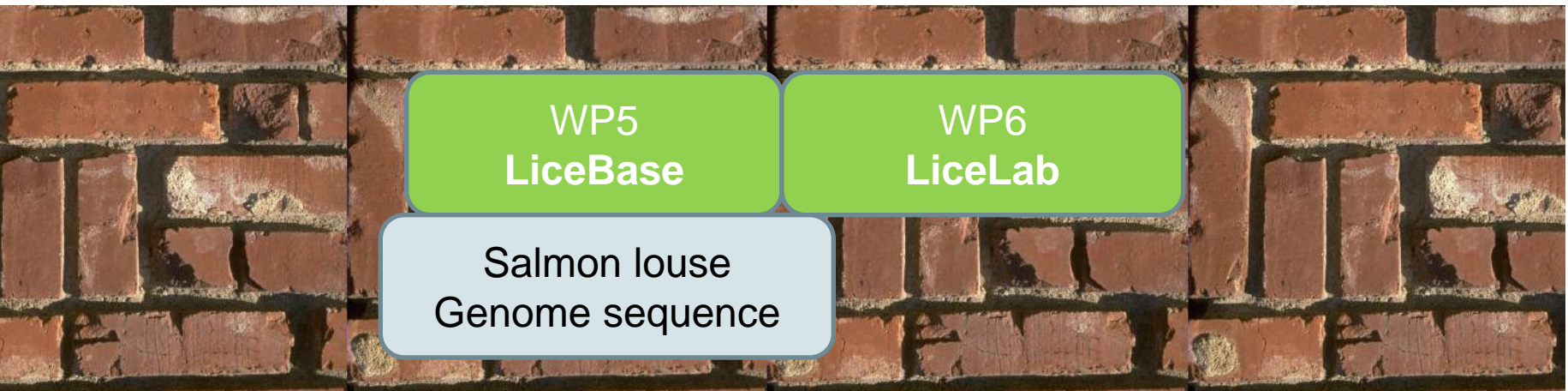
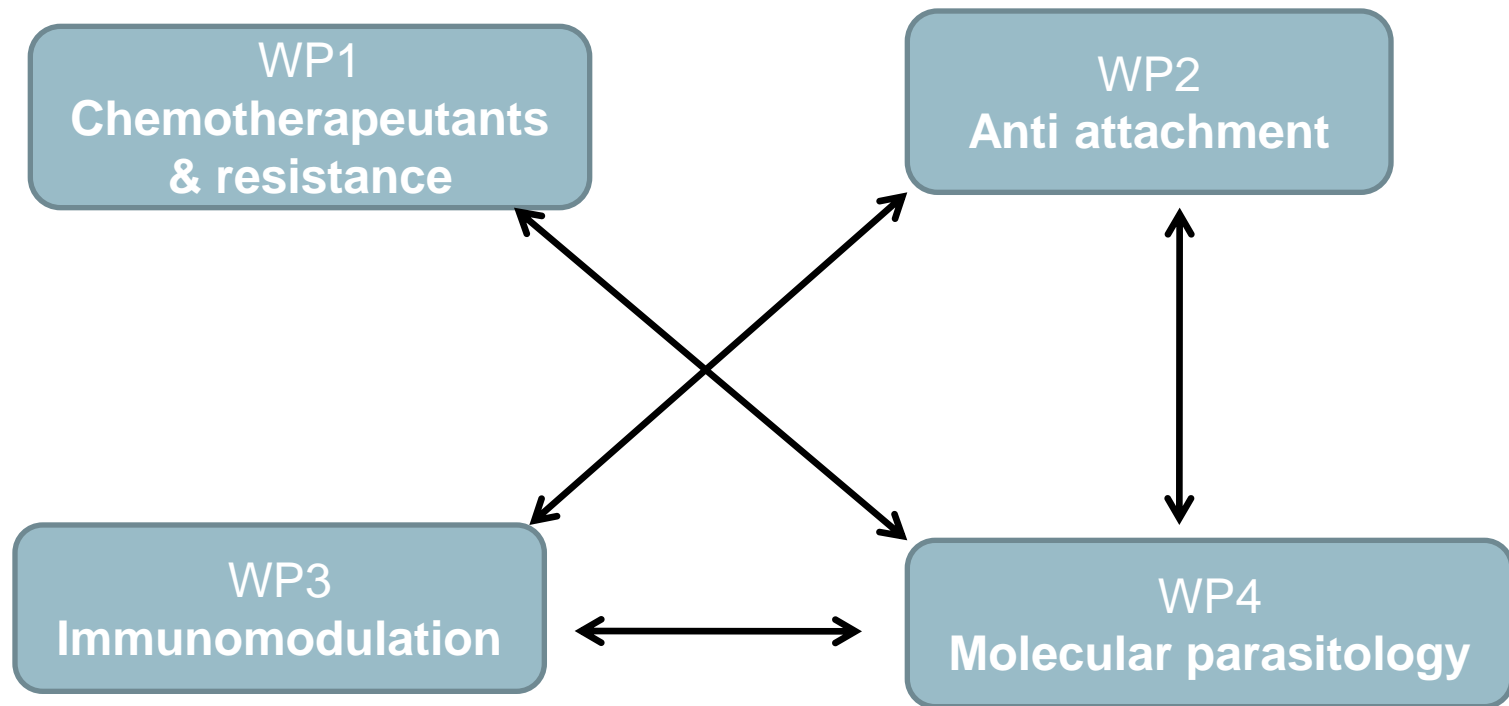


Dr. Sussie Dalvin



Lars Hamre

Interaction of the different WPs





Improved Sea Lice control

**Salmon louse
biology**

**New
Medicines**

**Feed
supplements**

**Innovation
for
Improved Lice control**

**Salmon louse
genome**

**Immune
control**

**Wet-lab
facility**



Salmon louse genome (WP5)

- Inbred strain
- High Throughput sequencing
- assembly
- ESTs + RNAseq
- annotation

LiceBase:
annotation & establish an integrative database resource

Benefits for SLRC-researcher

- Interesting features in salmon louse genome that help to understand its biology
- Easy to use tools which save an enormous amount of time-consuming work for molecular biologists

e.g. interesting gene known from other organism

⇒ blast against genome ⇒ yes/no ⇒ find genomic sequence, predicted EST, protein sequence, splice variants, orthologs, paralogs,

Or: finding genes not known from other organisms

WP5: Bioinformatics

- Determine full length sequence
- Copy number of candidate gene
- Analyze homologs in related organisms
- Determine gene repertoire for different molecule synthesis systems
- Map metabolic pathways
- Identify stage/organ specific transcripts (from RNA sequencing of selected stages and isolated organs)

Gene-tool: EnsemblMetazoa



e! EnsemblMetazoa | BLAST | Sequence Search | BioMart | Tools | Downloads

Lepeophtheirus salmonis | Location: LSalAtI2s877:193,176-229,870 | Gene: EMLSAG000000

e! EnsemblMetazoa | BLAST | Sequence Search | BioMart | Tools | Downloads

Search: for

e.g. CPR34 or chitin*

[Login/Register](#)

Lepeophtheirus salmonis
Lepeophtheirus salmonis

Provider [EnsemblGenomes](#) | Taxonomy ID [72036](#)

Search *Lepeophtheirus salmonis*...

e.g. [EMLSAG00000012167](#) or [LSalAtI2s877](#) or [synthetase](#)

Genome assembly: [L.SalAtI2s](#)

More information and statistics

Download DNA sequence (FASTA)

Comparative genomics

What can I find? Homologues, gene trees, and whole genome alignments across multiple species.

More about comparative analysis

Download alignments (EMF)

Popular genomes

- Caenorhabditis elegans**
WBcel235
- Drosophila melanogaster**
BDGP5
- Strongylocentrotus purpuratus**
GCA_000002235.2
- Anopheles gambiae**
AgamP3
- Nematostella vectensis**
ASM20922v1
- Bombyx mori**
Bmor1

[★ Log in to customize this list](#)

All genomes

ences, non-

Example gene

Example transcript

Ensembl Metazoa release 20 - September 2013 © [EBI](#)

Ensembl Genomes is developed by the European Bioinformatics Institute (Part of the European Molecular Biology Laboratory) EMBL-EBI and is powered by Ensembl software system for the analysis and visualisation of genomic data.

- Hymenoptera
 - Apis mellifera
 - Atta cephalotes
 - Nasonia vitripennis
- Lepidoptera
 - Bombyx mori
 - Danaus plexippus
 - Heliconius melpomene
- Coleoptera
 - Tribolium castaneum
- Hemiptera
 - Acyrthosiphon pisum
 - Rhodnius prolixus
- Phthiraptera
 - Pediculus humanus
- Crustacea
 - Daphnia pulex
- Myriapoda
 - Strigamia maritima
- Chelicerata
 - Ixodes scapularis
 - Tetranychus urticae
- Echinodermata
 - Strongylocentrotus purpuratus
- Annelida
 - Capitella teleta
 - Helobdella robusta
- Mollusca
 - Crassostrea gigas
 - Littorina saxatilis

- genomic sequence
- EST
- protein sequence
- splice variants
- Orthologs/paralogs
- ...

Gene-tool: EnsemblMetazoa

e! EnsemblMetazoa | BLAST | Sequence Search | BioMart | Tools | Downloads | Help & Documentation

Lepeophtheirus salmonis | Location: LSalAtI2s877:193,176-229,870 | Gene: EMLSAG00000012167 | Trans: EMLSAT00000012167

Gene-based displays

- Gene summary
- Splice variants (1)
- Transcript comparison
- Supporting evidence
- Sequence
- External references
- Regulation
- Expression
- Ontology
- Metazoan Compara
 - Genomic alignments (image)
 - Genomic alignments (text)
 - Gene tree (image)
 - Gene tree (text)
 - Gene tree (alignment)
 - Gene gain/loss tree
 - Orthologues (41)
 - Paralogues (25)
- Pan-taxonomic Compara
 - Gene Tree (image)
 - Gene Tree (text)
 - Gene Tree (alignment)
 - Orthologues
- Phenotype
- Genetic Variation
 - Variation table
 - Structural variation
 - Variation image
- External data
 - Personal annotation
- ID History
 - Gene history

Gene: EMLSAG00000012167

Description maker-LSalAtI2s877-snap-gene-1.30


Location [SuperContig LSalAtI2s877: 193,176-229,870](#) forward strand.

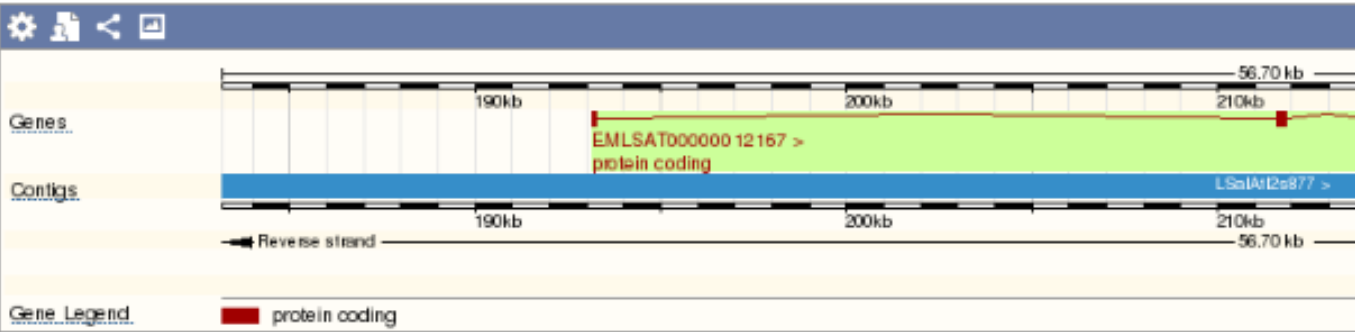
Transcripts This gene has 1 transcript (splice variant) [Show transcript table](#)

Gene summary i

Gene type Protein coding

Prediction Method Genebuild by [EnsemblGenomes](#).


Go to Region in Detail for more tracks and navigation options (e.g. zooming)



The visualization shows a genomic region from 190kb to 210kb. A green bar represents the gene EMLSAT00000012167, which is protein coding. Below it, a blue bar represents the contig LSalAtI2s877. A legend indicates that red bars represent protein coding regions. A 'Reverse strand' arrow is shown at the bottom left.

i **Configuring the display**

Gene tree



Gene-based displays

- Gene summary
- Splice variants (1)
- Transcript comparison
- Supporting evidence
- Sequence
- External references
- Regulation
- Expression
- Ontology
- Metazoan Compara
 - Genomic alignments (image)
 - Genomic alignments (text)
 - Gene tree (image)**
 - Gene tree (text)
 - Gene tree (alignment)
 - Gene gain/loss tree
 - Orthologues (16)
 - Paralogues
- Pan-taxonomic Compara
 - Gene Tree (image)
 - Gene Tree (text)
 - Gene Tree (alignment)
 - Orthologues
- Phenotype
- Genetic Variation
 - Variation table
 - Structural variation
 - Variation image
- External data
 - Personal annotation
- ID History
 - Gene history

Configure this page

Add your data

Export data

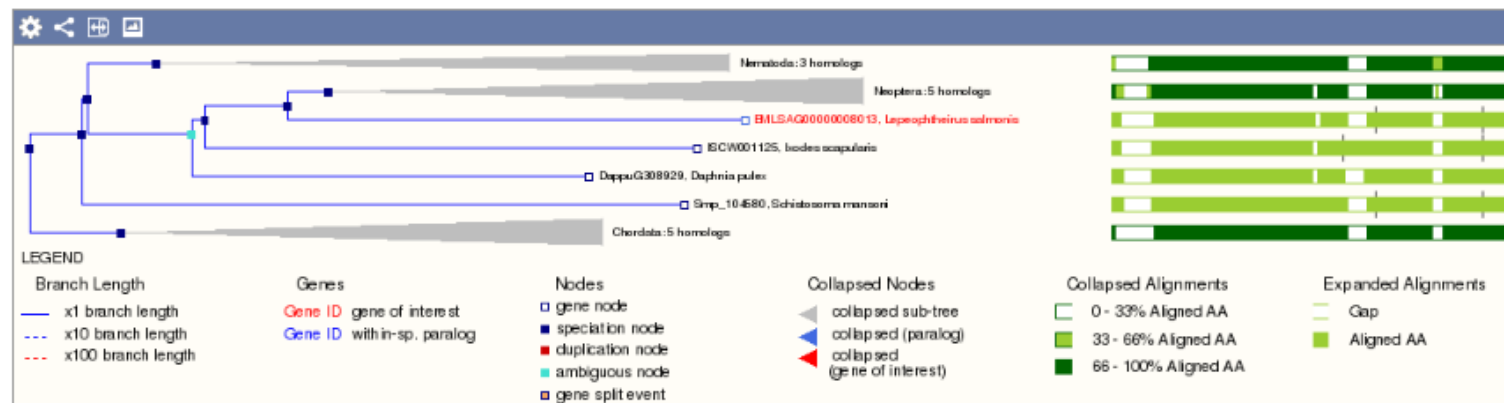
Gene: EMLSAG00000008013

Description	augustus_masked-LSalAtI2s476-processed-gene-1.0
Location	SuperContig LSalAtI2s476: 138,258-139,136 reverse strand.
Transcripts	This gene has 1 transcript (splice variant) Show transcript table

Gene tree (image)

 GeneTree [EMGT00050000016967](#)

Number of genes	17
Number of speciation nodes	14
Number of duplication	1
Number of ambiguous	1
Number of gene split events	0



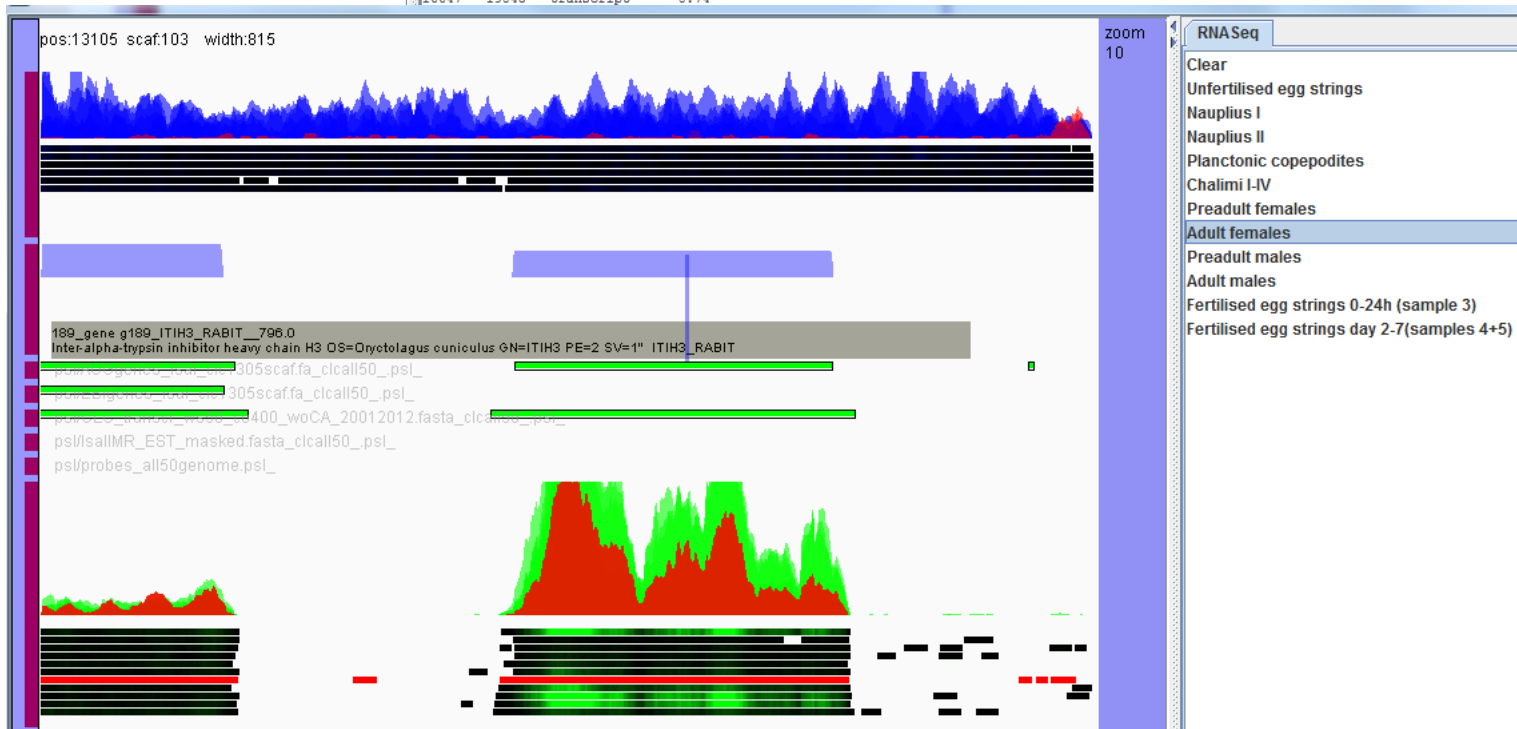
RNA-sequencing

- Shows in which stages interesting genes are expressed
- Helps to get more information about unknown genes

SHOW PFAM info : pfam.sanger.ac.uk trypsin

	contig	gene	length	start	end	strand	test	SWISSPROT	Gene	BLAST_hit	BLAST_hit_short	hs
188	clcAll50s103	gene g189	798	16647	19043	-	189	Q9GLY5	ITI13_RABIT	*gnl BL_O...	inter-alpha-trypsin i...	
688	clcAll50s430	gene g689	223	7468	8389	-	689	P07478	TRY2_HU...	*gnl BL_O...	trypsin-2 os=homo...	
1838	clcAll50s12...	gene g1839	515	2412	4072	+	1839	Q5G266	NETR_TRA...	*gnl BL_O...	neurotrypsin os=tra...	
2342	clcAll50s16...	gene g2343	267	70210	72290	-	2343	P35005	TRYE_DR...	*gnl BL_O...	trypsin epsilon os=d...	
2413	clcAll50s16...	gene g2414	270	18698	19510	+	2414	P35038	TRY4_ANO...	*gnl BL_O...	trypsin-4 os=anoph...	
2667	clcAll50s18...	gene g2668	265	34607	35593	-	2668	P00765	TRYP_AST...	*gnl BL_O...	trypsin-1 os=astacu...	
2961	clcAll50s21...	gene g2962	248	89376	91891	-	2962	P00765	TRYP_AST...	*gnl BL_O...	trypsin-1 os=astacu...	

gene g189 clcAll50s103
16647 19043 gene 0.74
16647 19043 transcript 0.74



Wet Lab facilities at SLRC (WP6)

1x1 meter tanks

- designed to maintain lice strains as
 - material for research general
 - test efficacy of treatments: RNAi, vaccines, drugs...



Wet Lab facilities at SLRC (WP6)

Single fish tanks

- Efficacy assays (vaccine, drugs, feed)
- Selective breeding
 - monitor loss of lice from individual fish
 - screening of many RNAi targets using a minimum of fish and space



Wet Lab facilities at SLRC (WP6) Hatchery (flow through)



- Wet table with 100 outlets
- Room for 1600 32mm incubators (each labeled with unique identity)



<http://www.slrc.no/sample-page/facilities/licelab-design/>

Wet Lab facilities at SLRC (WP6) Additional capacity at ILAB and BIO



- 500 litre tanks



Wet Lab facilities at SLRC (WP6)



LiceLab at UIB

- 10 1x1 meter tanks
- 114 single fish tanks (improved design)
- Hatchery (flow through); room for 1600 32mm incubators
- additional capacity at ILAB and BIO when required (500 litre tanks)

LiceLab at IMR

- 48 single fish tanks
- Hatchery: incubators can be run simultaneously with variable salinity and temperature (flow through)
- 160 litre, 250 litre and 500 litre fish tanks: salinity and temperature can be mixed in each individual tank.

LiceLab EWOS Dirdal

- 16 tanks (500 litre)
- Hatchery (stagnant water)

Lice material (WP6)



- 6 strains of lice maintained
- resistant strain

Why more lice when there are so many lice around?

- ⇒ Availability of lice from right stage at right time assured
- ⇒ Comparable experimental material
- ⇒ Enough experimental material at given time point

RNA interference (WP6)

A process in which the introduction of double-stranded RNA into a cell inhibits the expression of genes.

(naturally or **artificially introduced**)

- Sequence specific (choose gene to be silenced)
- Silencing lasts from 2 days to at least 2 month

⇒ Information about the function of a gene

⇒ **Simulation of vaccine or drug: targets**



RNAi screening: Finding targets (WP6)

Goal: identify genes that encode proteins that are essential and unique!

Salmon louse genome

- ~13.000 genes. Screen of 100 genes/year (2012, 2013)

Sequence analysis

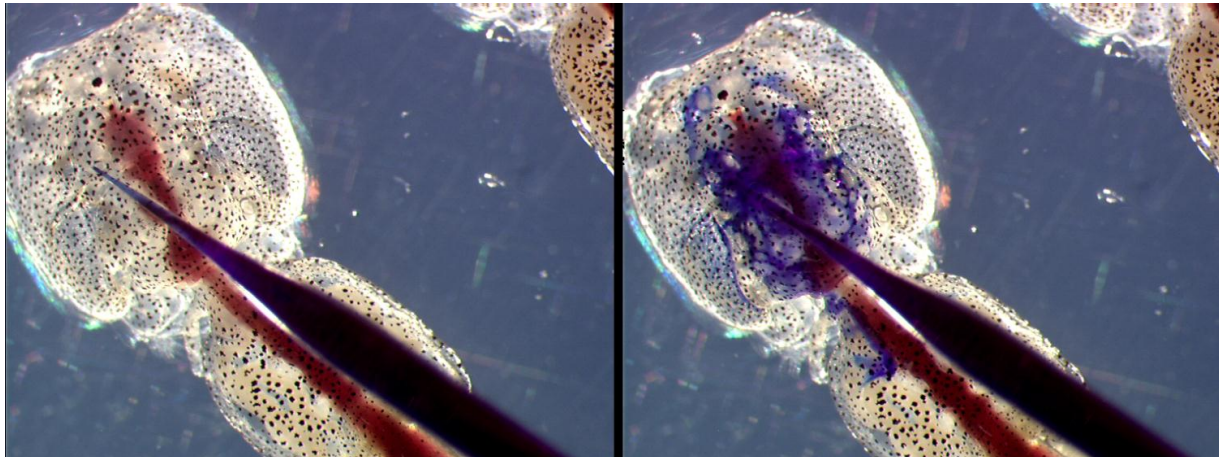
- Exclude conserved genes
- Look for secreted proteins
- Unknowns (30-40% have little or no information)

Expression patterns (RNA-seq)

- Selection based on when proteins are used

Preadult/adult RNAi screen

- collect preadult lice (timed infection)
- Injection of dsRNA
- Lice on fish (single fish tanks)
- Sample adult lice: investigate and incubate eggs
- Investigate offspring

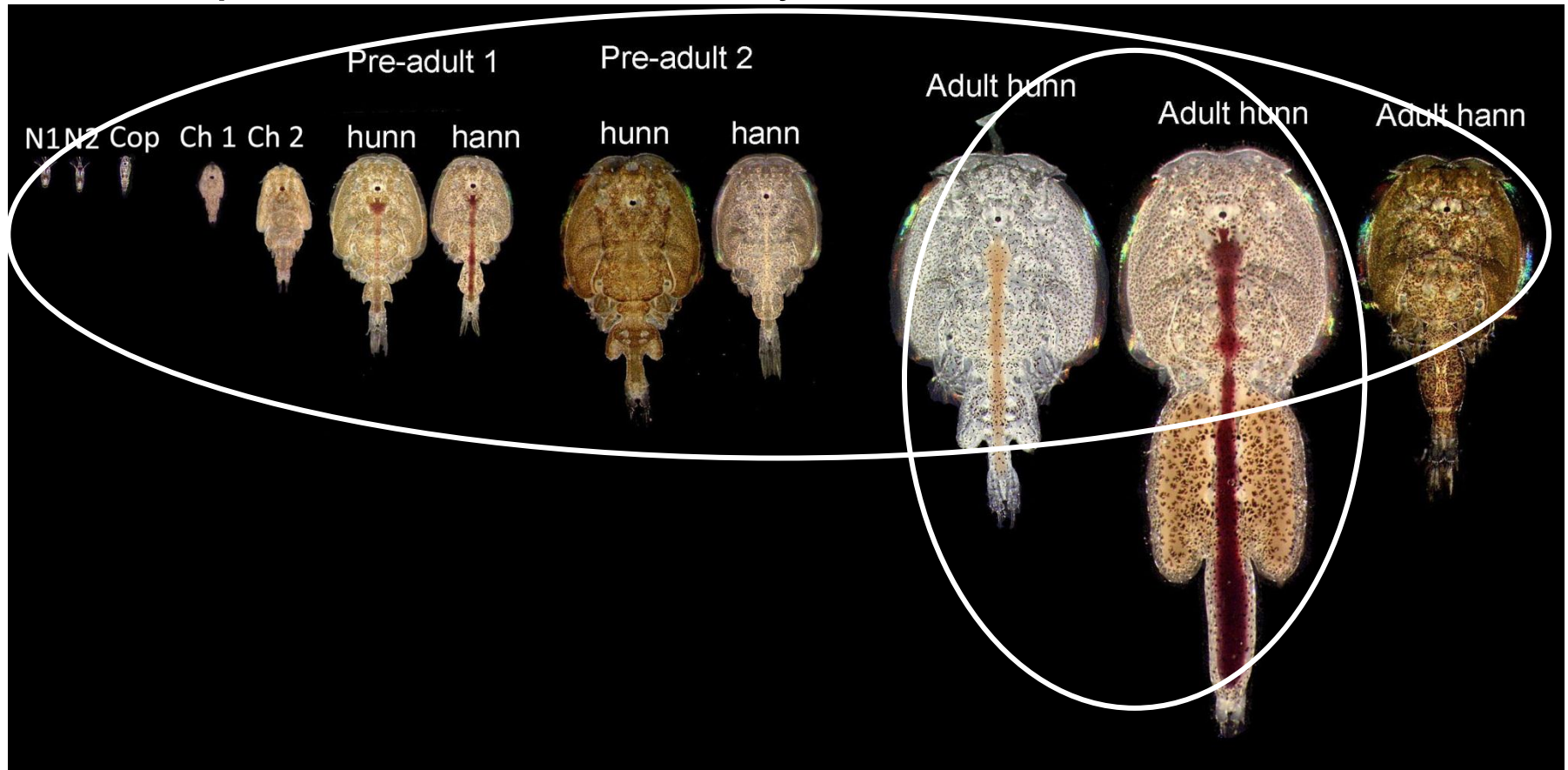


- 30 with abnormal phenotype
- Four candidate genes to be produced for test vaccines (Novartis)

Effective RNAi methode for nauplia screen

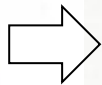
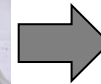
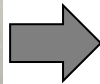
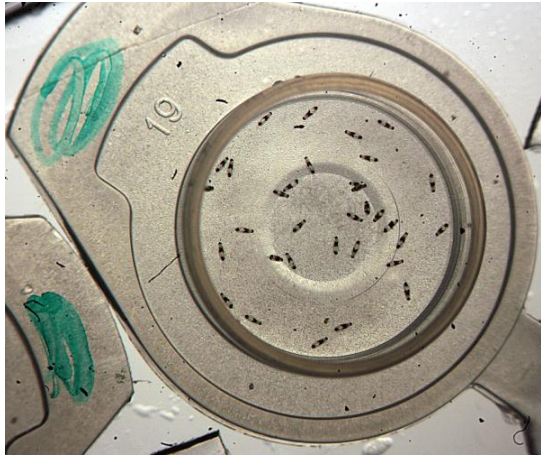


- Preadult/adult screen: labour intensive, small part of the life cycle
- Nauplia screen: whole lifecycle, both sexes



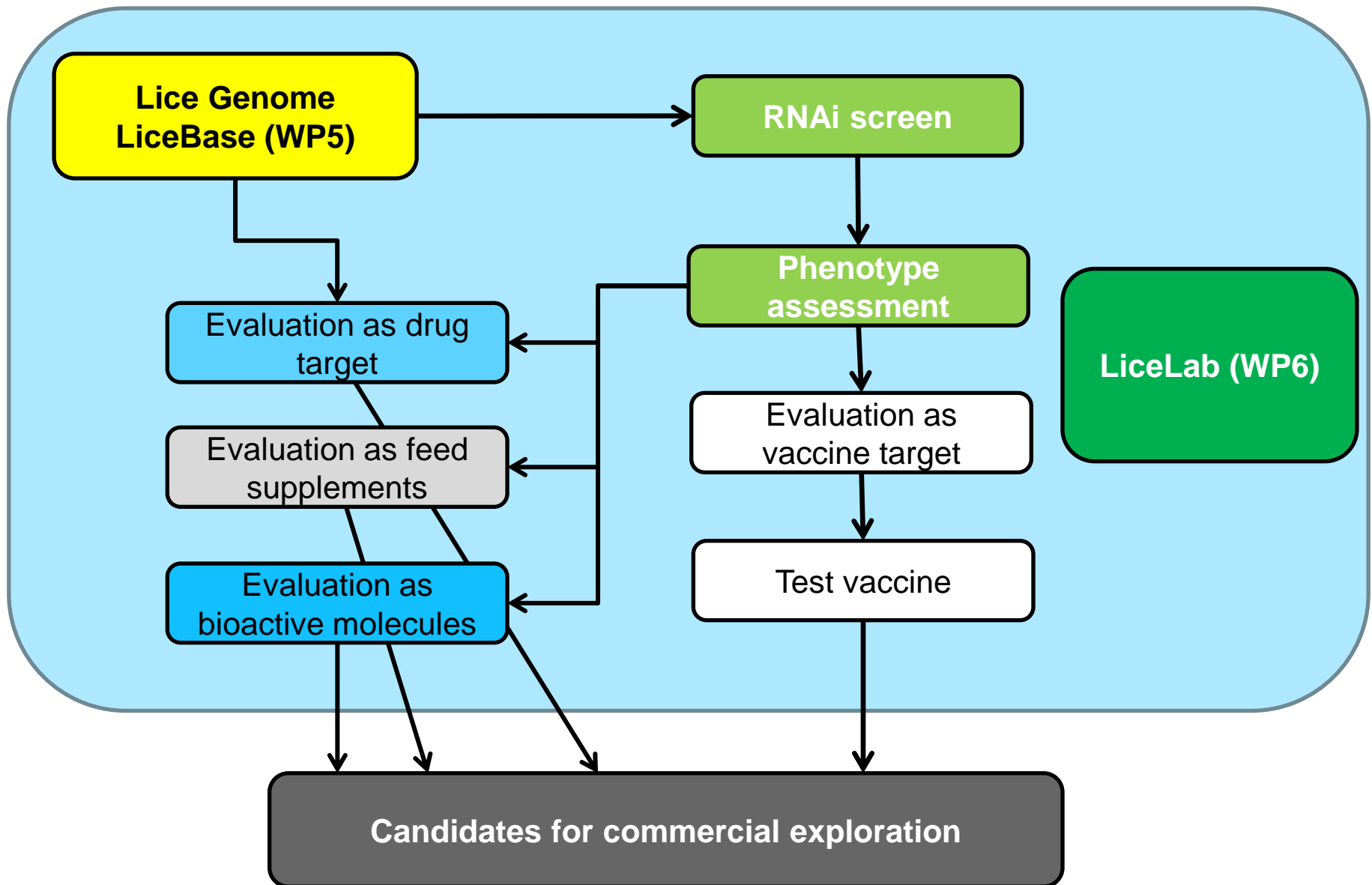
Nauplius screen

- Incubation in double stranded RNA during molt



- Successful silencing
- nonviable phenotype
- Longevity is in test now

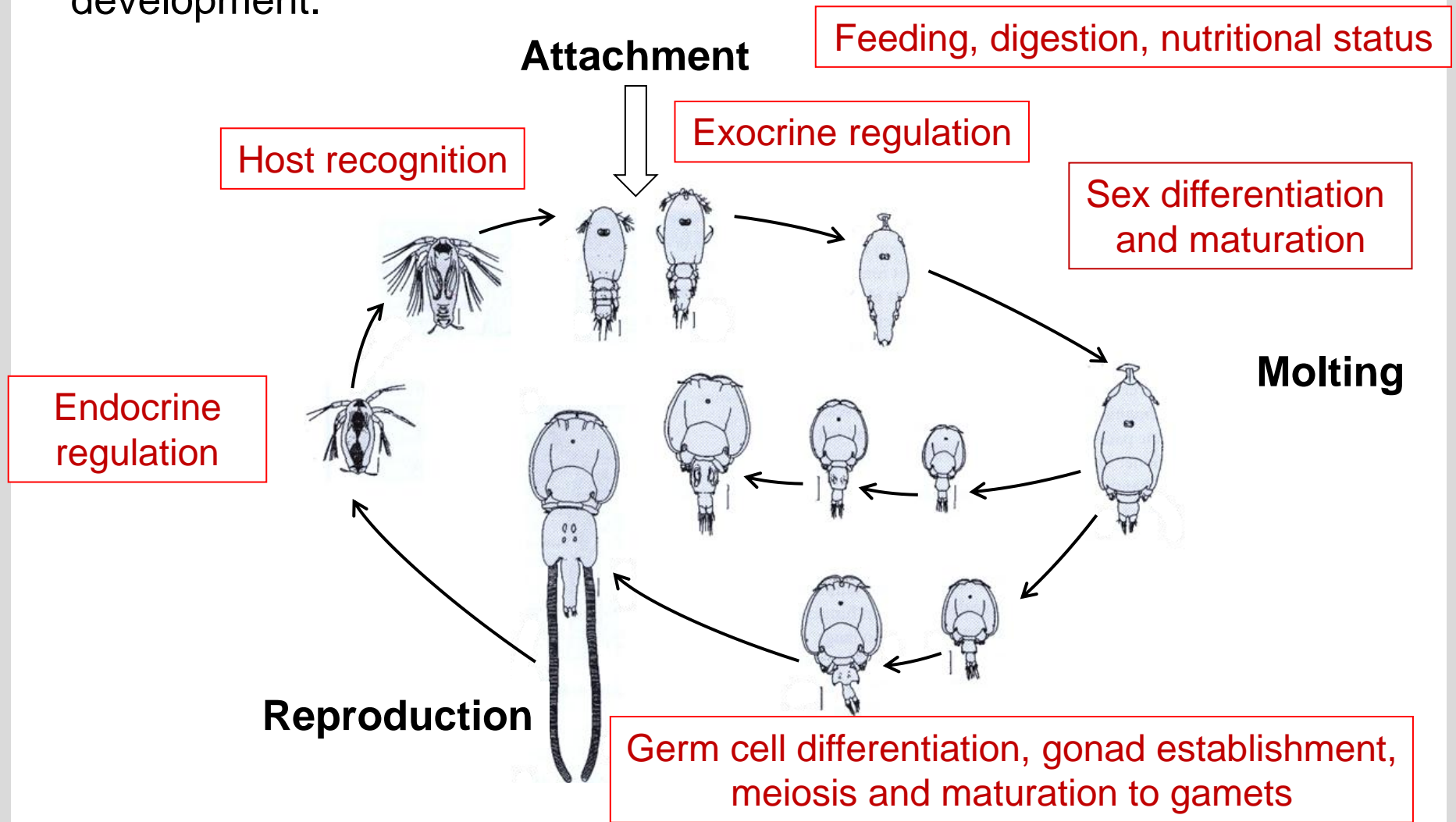
Candidate gene screening





WP4 Molecular parasitology and host parasite interactions:

Identification and evaluation of targets for immune control and vaccine development.



WP4 Molecular parasitology and host parasite interactions:

Identification and evaluation of targets for immune control and vaccine development.

- Copepodid biology
 - Chemosensory system
 - Gene regulation in parasitic copepodids
- Reproduction, germ cell differentiation and maturation
 - Nuclear receptors
 - Germline formation
 - Oocyt maturation
- Endo and exocrine system in salmon louse

Finding ligands of receptors:

- **Ligand:** Any substance (e.g. hormone, drug, functional group, etc.) that binds specifically and reversibly to another chemical entity to form a larger complex. A ligand may function as agonist or antagonist.

Receptors

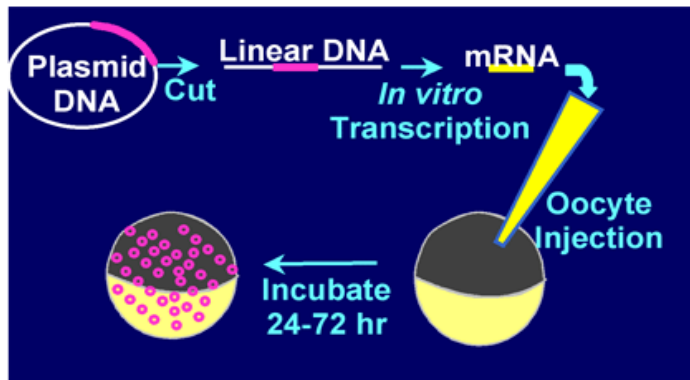
- chemosensory system (attraction/repulsion)
- Endocrine system (hormones; metabolism, growth and development)

How to test possible ligands?

- Xenopus oocyte system (Novartis, Saint Aubin)
- Two-hybrid Ligand activation assay

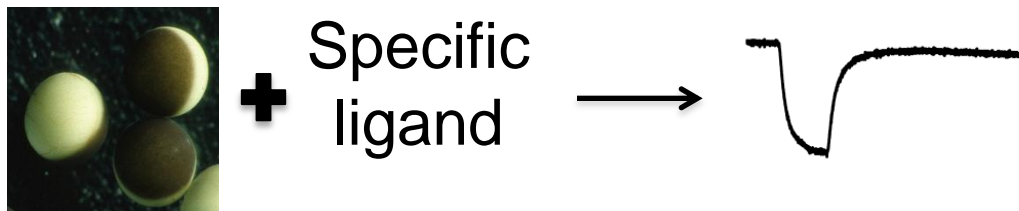
Sensory system of copepodites (WP4 & WP2)

- Xenopus oocyte system (Novartis, Saint Aubin)



Finding substances that could interrupt the infestation of the copepodites

Voltage measurement

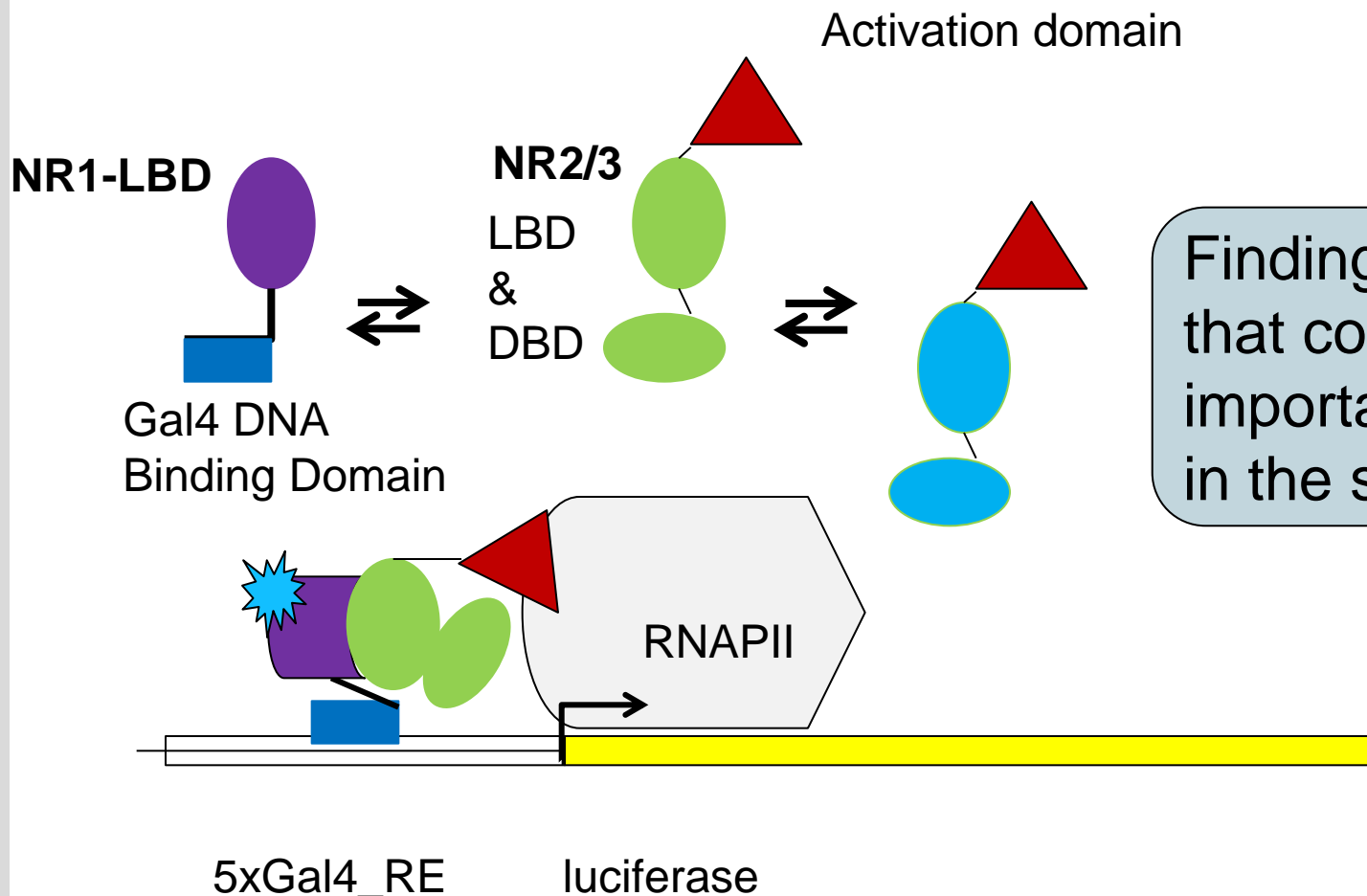


- 27 ligands tested (Different concentration, time)

Hormone receptor ligands

Hormones: Molting, Reproduction

- Two-hybrid Ligand activation assay

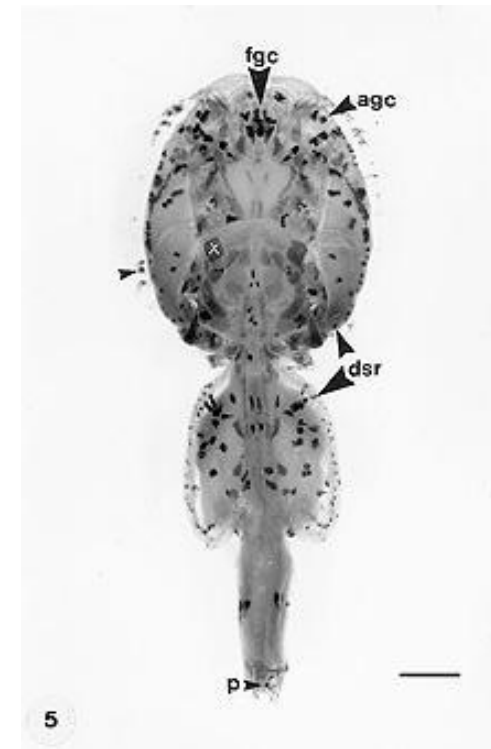
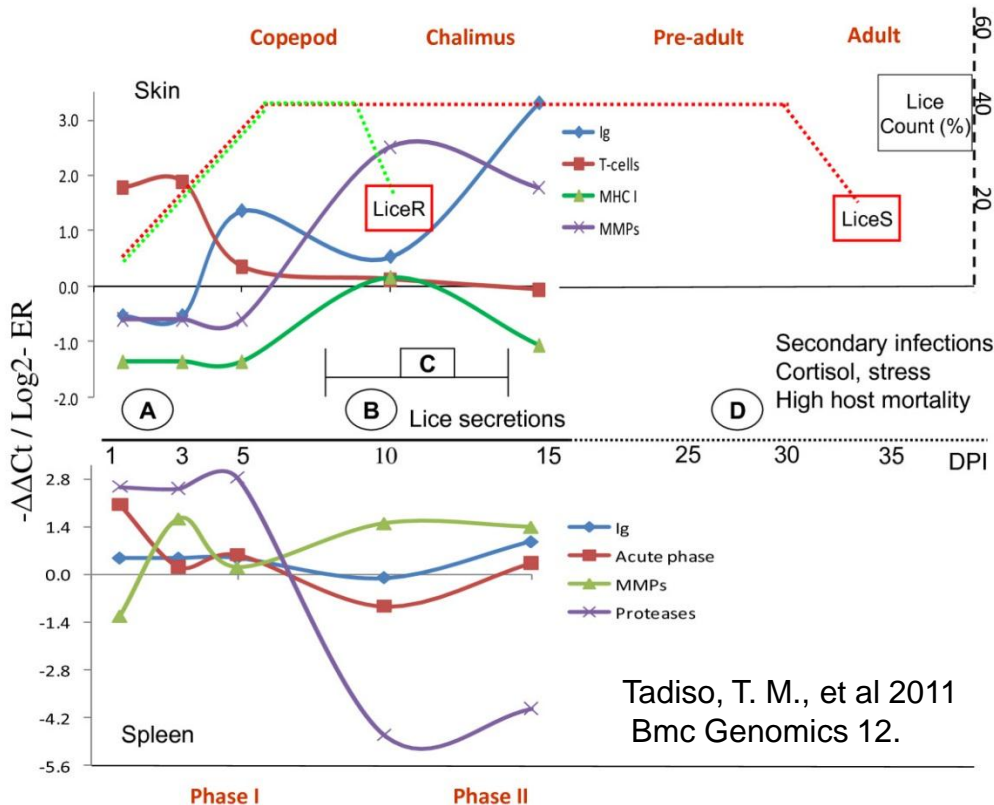


Finding substances that could interrupt important processes in the salmon louse



Salmon lice glands

- Numerous glands
- Different morphology
- Modulation of host response?



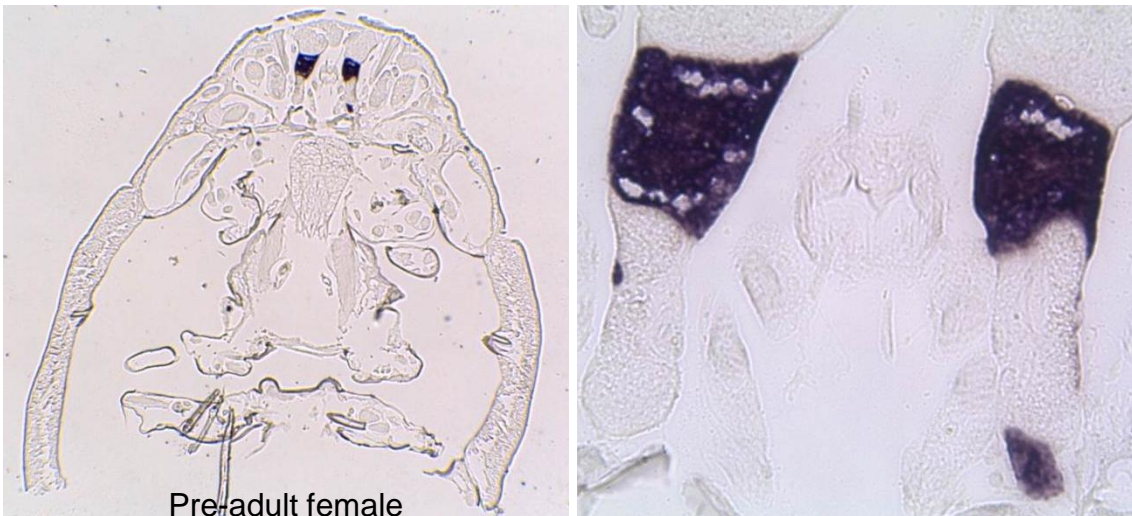
Bell et al. 2000
DAB-staining -
exocrine glands

- Possible target for vaccines/new drugs

Tadiso, T. M., et al 2011
Bmc Genomics 12.

Finding genes expressed in this glands

- Finding candidate genes in the genomic resources starting from immunomodulating substances known in other organisms
- Localization in the salmon louse by *in situ* hybridisation



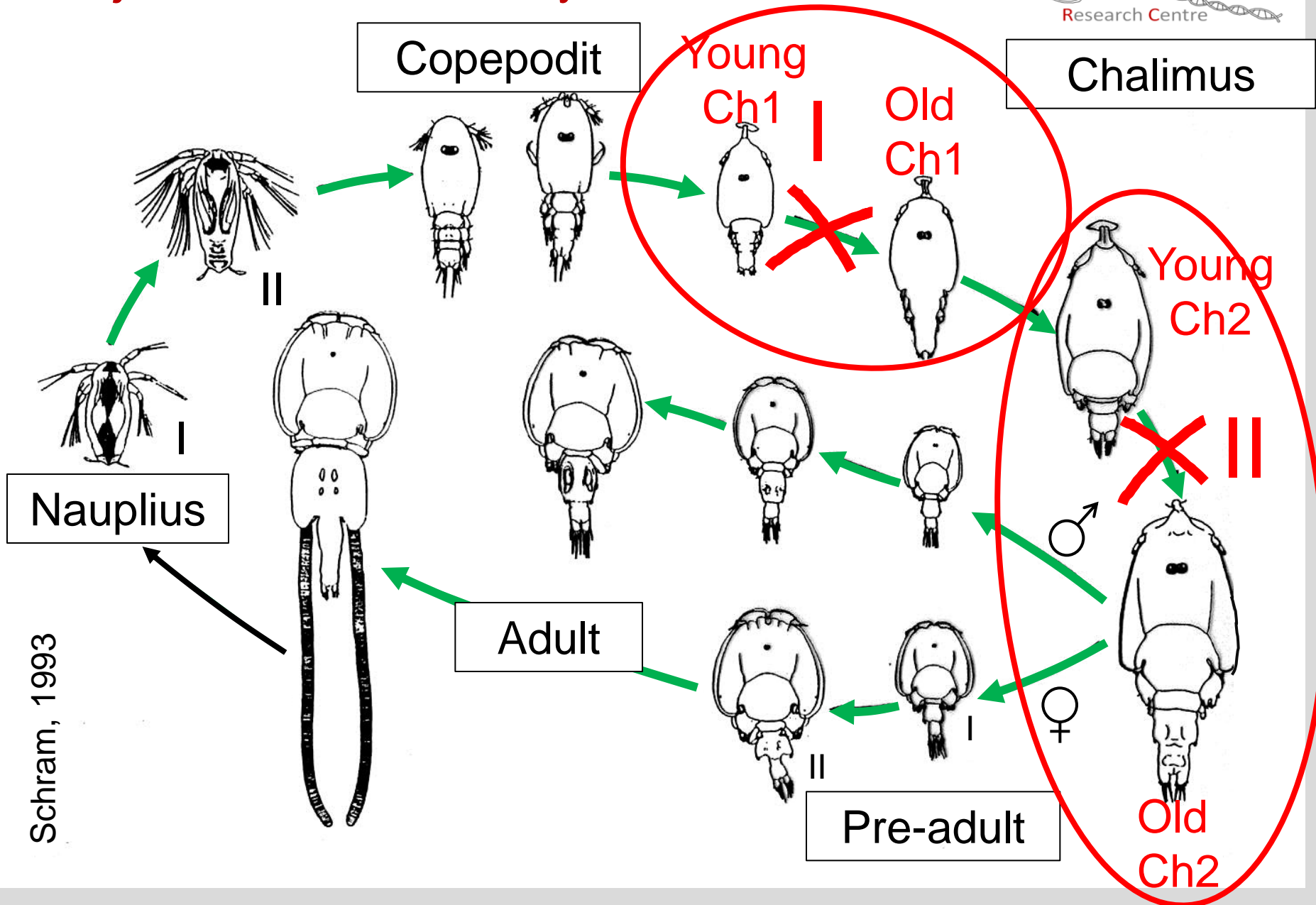
- RNAi with candidate
- Less infestation???

Life cycle of the salmon louse

Wet-lab facilities gave possibilities for inspecting the life cycle of the salmon louse in a new way

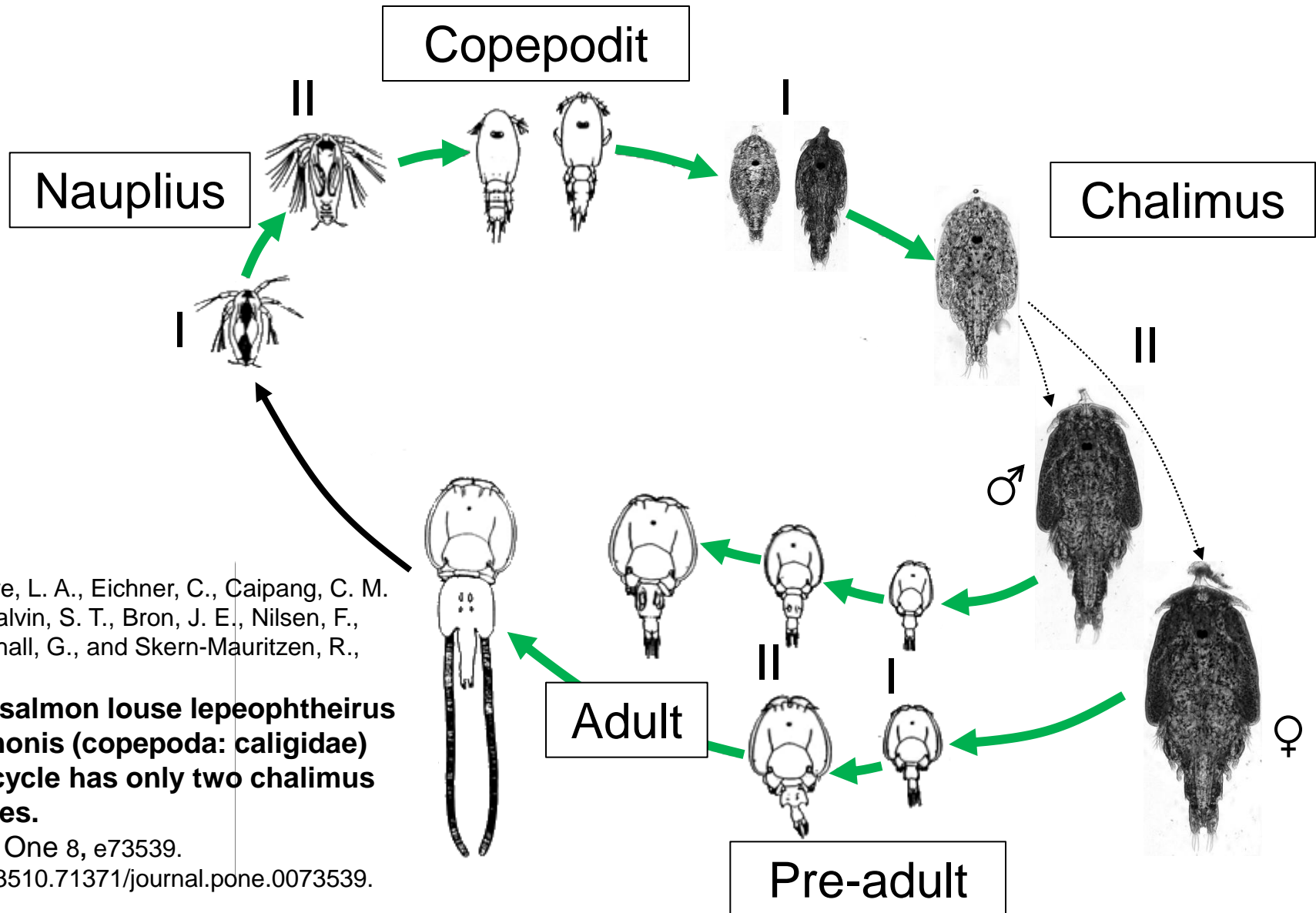
- Investigation of development and molting in early infested stages is impossible by sampling “wild” lice.
- Flow-through system: survival and molting of lice off the fish possible; observation of individual lice in single incubators

Adjustment of the life cycle of *L. salmonis*



Schram, 1993

New life cycle of *L. salmonis*



Hamre, L. A., Eichner, C., Caipang, C. M. A., Dalvin, S. T., Bron, J. E., Nilsen, F., Boxshall, G., and Skern-Mauritzen, R., 2013.

The salmon louse *lepeophtheirus salmonis* (copepoda: caligidae) life cycle has only two chalimus stages.

Plos One 8, e73539.

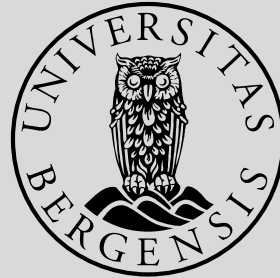
doi:73510.71371/journal.pone.0073539.

Conclusions

- WP5: LiceBase (genome resources/ integrated database)
 - ⇒ The whole genome is sequenced, assembled and annotated ⇒ available for SLRC-researcher and soon be published
- WP6: LiceLab
 - ⇒ Wet-lab facilities are expanded and improved for better reproducibility and higher turnover
 - ⇒ Candidate genes to be produced for test vaccines identified by RNA interference screening
 - ⇒ screening method for nauplia screen established (whole lifecycle, both sexes, higher throughput)

Conclusions

- WP4&2 (Molecular parasitology, Antiattachment)
 - ⇒ progress in investigation of the endocrine, exocrine and sensory system (foundation for target search).
- WP1 (Medicine & Resistancy) ⇒ Prof. Tor. E. Horsberg
- WP3 (Immunomodulation) (NVH)
 - ⇒ investigation of immunomodulation on the fish site in cell culture
 - ⇒ experiments on fish at LiceLab facilities going on as well



University of Bergen | Department of Biology