Correlations between gene expression and epigenetic markers as a result of single and multiple stressor exposure induction, studied in zebrafish founder and offspring generations.
Epigenomics

Histone marks

5mCpG

DNA methylation

RNA

Transcriptomics
Model organisms

**Mammalian Models:**
- Mouse
- Rat

**Non-Mammalian Models:**
- *S. cerevisiae* (budding yeast)
- *S. pombe* (fission yeast)
- Neurospora (filamentous fungus)
- *D. discoideum* (social amoebae)
- *C. elegans* (round worm)
- Daphnia (water flea)
- *D. melanogaster* (fruit fly)
- *D. rerio* (zebrafish)
- Xenopus (frog)
- Gallus (chicken)

**Other Model Organisms:**
- Arabidopsis

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### The ICRP Reference Animals and Plants

<table>
<thead>
<tr>
<th>Organism group</th>
<th>Species</th>
<th>Life stage</th>
<th>Ecosystem/Human model organism</th>
<th>Availability / expertise</th>
<th>ICRP RAPs</th>
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<tr>
<td>Plants</td>
<td>Tall clover (Arabidopsis thaliana)</td>
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<td>Terrestrial</td>
<td>UMB</td>
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<td>Lemma sp</td>
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<td>NIVA</td>
<td>no</td>
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<td>Algae</td>
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<td>Rainbow trout (Oncorhynchus mykiss)</td>
<td>In vivo studies with early life stages?</td>
<td>Freshwater</td>
<td>NIVA</td>
<td>No</td>
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<tr>
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<td>Freshwater</td>
<td>NIVA</td>
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<tr>
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<td>Freshwater</td>
<td>NIVA</td>
<td>no</td>
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</tbody>
</table>
Zebrafish is an excellent lab animal:

- a robust small size aquarium fish with short life cycle (4 generations/year)
- a transparent embryo which develops in a dish of water.
- hatches day 3, starts feeding day 6
- embryo development well characterized
- the genome fully sequenced and annotated
- multigenerational studies
- keeping and monitoring a defined and optimized environment
- mutants, advanced GM methods and tg models
- low cost
- 3R-oriented
Centre for Environmental Radioactivity (CERAD) 2013-2022

gamma UV multiple stressors

RA3 Milestones (application)

SINGLE STRESSOR EXPOSURES
GAMMA & UV EXPOSURES
CLIMATE CONDITIONS
MULTIPLE STRESSOR EXPOSURES

Centre for Environmental Radioactivity (CERAD) 2013-2022
Early life stage effects - zebrafish model

- **RNA-seq** uncovers the complete transcriptome
- **MeDIP-seq** uncovers the DNA methylome
- **ChIP-seq** uncovers mechanisms of the epigenome
Correlation between the transcriptome and epigenome => gene expression predictions
Already before zygotic genome activation (ZGA) >1000 developmentally-regulated promoters are marked with the activation mark H3K4me3

Lindeman et al. 2011 Dev Cell
non-exposed genome wide transcriptome

multi-stressor exposed genome wide transcriptome

compare

Lindeman et al. 2011 Dev Cell
ncRNAs
thousands of small and long non-coding RNAs during zebrafish embryogenesis are largely unknown .................... Fish-miR project 2013-2015 aims at RNA-seq characterization with functional studies (UiN, UiT, NVH, Oregon Univ)

zebrafish miRNAs:

- miRNAs (22nt): 740 in the Ensembl annotation
- piRNAs (25–30nt): $10^5$ (Wei 2012)
- other ncRNAs: 225 tRNA (73–93nt) 3’ or 5’end derived RNAs of 18–28nt (see 30–34nt mse-tsRNA in sperm and oocytes doi:10.1038/cr.2012.181)
- addition of non-template nt’s affects transcript stability: 3’A ($t_{1/2}$ up) and U ($t_{1/2}$ down)
- 70% expressed at low levels

Wei et al. 2012. RNA 18, 915–929,
Aanes, Collas, Alestrom, Review subm. to Briefings in Functional Genomics
Multi-Ztress Submitted Aug 14: 2 year postdoc (Cecilia Winata, GIS)

Figure 1: World capture fisheries and aquaculture production FAO 2012 Report, ISSN 1020-5489, published at www.fao.org/docrep/016/i2727e/i2727e.pdf

Figure 2. Overview over planned exposures and analyses for the proposed project. Horizontal arrows represent zebrafish generations of 6 month, with puberty around 3 months of age. Founder generation (F0) and progeny generation (F1) are indicated. Vertical arrows represent exposures (gray) and analyses (yellow). The inserted frame on top represents the early developmental stages previously characterized by the applicant and the host laboratory (11-19). "RNA" and "ChIP" symbolizes Next generation sequencing of transcriptomes and epigenomes.
Effect of UV exposure on farmed fish

• In Europe most aquaculture is based on intensive sea cage production (salmon, Atlantic cod, Atlantic halibut, seabass, seabream)
• Such intensively raised fish are exposed to unnaturally high levels of UV exposure.
• It has been demonstrated that increased exposure to UV-B radiation has negative effects on growth, condition and immune function in juvenile Atlantic salmon.
• Excessive UV exposure has also been reported to result in “summer lesion syndrome” in Atlantic salmon, with affected fish showing lesions in the skin behind the pectoral fins, rapidly deepening to involve the muscle layers.
• Also, it has been suggested that increased UV exposure is one of the causal factors for the increased levels of fin rot and cataracts reported in farmed Atlantic salmon.
• These abnormalities are both a major production and welfare concern to the aquaculture industry.
• The objective of this CERAD RA5 study is to investigate whether high UV exposure can increase the incidence of a number of common production abnormalities in farmed salmon, including skin lesions, fin rot and cataracts.
• Further the effect of high levels of UV exposure on growth rates and immune function (plasma IgM concentration and lysozyme activity) will be investigated.
Provisional work plan

- Assessment of morphological effects of UV exposure on early embryos
- 1st first generation zebrafish embryos (F0) exposed to low dose of UV radiation
- Second generation zebrafish embryos (F1) indirectly exposed to UV radiation
- Perform transcriptome profiling with RNA-seq and epigenome profiling with ChIP-seq
- Analysis of next generation sequencing data
- Validation experiments and functional analysis of several identified target genes
- Arrangement of future activities, including grant applications aiming at sub-goal 3 (Use the results to predict and test effects on juvenile/adult zebrafish and Atlantic salmon)
- At least two publications in an international peer-reviewed journals
- Presentation of findings in at least 1 international conference (EZM 2015)
ongoing exposure experiments
gamma at Figaro
UV at NVH by NRPA

**Gamma:** 0, 0.4, 1, 4, 16 and 40 mGy/h for 24, 72, 120 h. Jan, Vidar, Hans-Christian, Ole-Christain, Terje, Thomas, Leonardo, Peter, Ana

**UV:** UVB 1, 3, 10 min. Terje, Thomas, Ellen

**RNA-seq** and **ChIP-seq** of ZGA stage zebrafish embryos with 0.4, 1, 4, 16 and 40 mGy/h gamma and defined doses UV

<table>
<thead>
<tr>
<th>Study</th>
<th>Study start</th>
<th>No fish/embryos</th>
<th>Ethical clearance</th>
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<tbody>
<tr>
<td><strong>Gamma exposure</strong></td>
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<tr>
<td>Dose response 5 days embryo test</td>
<td>Aug. 12.</td>
<td>3000 embryos</td>
<td>Not demanded</td>
</tr>
<tr>
<td>Three generation study</td>
<td>Aug. 19.</td>
<td>160 adult fish 80 m/80 f</td>
<td>Need application UMB and NVH</td>
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<tr>
<td>RBE 21 days exposure study</td>
<td>Aug. 27.</td>
<td>360 adult males</td>
<td>Application sent UMB</td>
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<td><strong>UV exposure</strong></td>
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<td></td>
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</tr>
<tr>
<td>Dose response 5 days embryo test</td>
<td>Depend on establishment of zebrafish box (August/September)</td>
<td>500 embryos pilot1 500 embryos pilot2</td>
<td>Not demanded</td>
</tr>
<tr>
<td>Zebrafish melanoma model</td>
<td>October</td>
<td>500 embryos?</td>
<td>Need application</td>
</tr>
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</table>
Zebrafish cardiac cycle is an important parameter to verify the toxicity effect of a compound or to check physiological parameters of a mutant. The ZebraLab software enable to automate this task and various experiment involving movement of organs (such as the gut transit).

Information request
IN Cell Investigator
Zebrafish Analysis
GE Healthcare Life Sciences

Summary
- IN Cell Analyzer 2000 was used for automated imaging of zebrafish in well plates, with fluorescence and transmitted light modalities.
- The system's large CCD camera under the 2X objective captures a field of view 7.6x7.6 mm². This enables rapid single image whole-well imaging from 96-well plates.
- The 2X objective has a lateral resolution of ~ 3.4 μm, far exceeding the need to capture detail even from small zebrafish organs, e.g. the heart, with a size ~ 300 μm.
- Higher resolution whole-well imaging is achieved with the system's field overlap functionality + automated image stitching with IN Cell Investigator 1.5.
- 3-Dimensional features of zebrafish were studied by automated Z-stacking.

Automated High Magnification Imaging of Zebrafish

Figure 1: Thumbnail of 5-dpf zebrafish images auto acquired on IN Cell Analyzer 2000. 2X objective, Corning Costar #3599, 96-well plate, 150 μl water/well. Upper 3 rows, transmitted light; lower 3 rows, FITC fluorescence channel.

Figure 2: Whole well transmitted light image of 5-dpf unstained zebrafish on IN Cell Analyzer 2000. 2X objective, autofocused, 96-well plate (Corning Costar #3599), 150 μl of water. Square image field of view (FOV), 7.6 mm; circular well diameter, 6.4 mm. Despite the large FOV, intensity nonuniformity from well center to edge is small and, if needed, can be easily corrected by flat field correction. Size reference bar is included during image capture.

Figure 3: Tail area of unstained 5-dpf zebrafish imaged with transmitted light modality on IN Cell Analyzer 2000. 10X objective, autofocused, 96-well plate as in caption to Fig. 1. Despite the near transparency of the larvae, fins, notochord, urogenital opening and pigmented cells are clearly visible. Size ref. bar is auto-captured.

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Length measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head width</td>
<td>U</td>
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<tr>
<td>Eye diameter</td>
<td>GH</td>
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<tr>
<td>Body length</td>
<td>AB</td>
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<tr>
<td>Pericardial edema indicator (PEI)</td>
<td>EF</td>
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<tr>
<td>Tail length</td>
<td>DB</td>
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<tr>
<td>Trunk length</td>
<td>CD</td>
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<tr>
<td>Abdominal width</td>
<td>KL</td>
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<tr>
<td>Notochord length</td>
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<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Area measurements</th>
</tr>
</thead>
<tbody>
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<td>Head</td>
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<tr>
<td>Eye</td>
<td>Black</td>
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<tr>
<td>Ear</td>
<td>Blue mesh</td>
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<tr>
<td>Heart</td>
<td>Medium green mesh</td>
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<tr>
<td>Liver</td>
<td>Red mesh</td>
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<tr>
<td>Swim bladder</td>
<td>Cyan mesh</td>
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<tr>
<td>Gastrointestinal tract</td>
<td>Light green mesh</td>
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<tr>
<td>Upper muscle</td>
<td>Yellow mesh</td>
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<tr>
<td>Notochord</td>
<td>Gray mesh</td>
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<tr>
<td>Lower muscle (tail)</td>
<td>Magenta mesh</td>
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</table>
Long term goal

early life stage mechanisms markers

mechanism specific tg reporter fish
NVH
Peter Aleström
Liqun Jenny Zhang (iPSC)
Håvard Aanes (Bioinform.)
Leonardo Martin (ncRNA)
Jan L Lyche (toxicogenomics)
Elisabeth LIE (Envir. tox)
Jan E Paulsen (Food tox)
Ian Mayer (Reprod. physiol)

Zebradish facility
Erik Ropstad (Head)
Jan R T Sørby (Facility man.)
Ana C. S. Tavara (Vet-technician)

CERAD-NVH
NVH in kind PhD student
PhD quote student

Univ. Oslo
Philippe Collas
Olga Østrup
Andrew H Reiner
Leif Lindeman
Ingrid S Andersen

Singapore (GIS)
S. Mathavan
Cecilia Winata

Singapore NUS
Zhiyuan Gong
NVH
In-kind: One senior researcher year
In-kind: NVH CERAD PhD stip 1 (2014-2016) - bioinformatics
In-kind: NVH-MatInf kvotestip (Sept 2013-Aug 2015) - zebrafish HTP screening

Søknader
In-kind: Marie Curie postdoc (submitted Aug 14) - transgenerational gene expression and epigenetics
In-kind: NVH CERAD PhD stip 2 (deadline 2. Sept) - transgenic reporter models

CERAD
Leif Lindeman: zebrafish and other species, epigenetics and small ncRNAs