



The Norwegian Zebrafish Platform



Zebrafish.no



Aleström Zebrafish Lab

The ZNN 2010 Conference and 2nd ZNN Annual Meeting

Thon Hotel Oslo Airport- Gardermoen, 29th -31st October 2010

To all participants

Welcome to the 2nd Zebrafish Network Norway (ZNN) meeting which consists of the ZNN 2010 Conference and the ZNN Annual Meeting 2010.

The ZNN Meeting co-functions as part of *zebrafish.no phd course 2010*.

The Organizing Committee

Cell phone:

Peter (+47) 92448644

Jan Roger (+47) 41212947



PROGRAM



ZNN2010

ZNN Conference 2010, October 29-31

ZNN Community meeting 2010 October 30

zebrafish.no phd course 2010 October 29 - November 5

1) Thon Hotel Oslo Airport October 29-31

2) NVH Campus Adamstuen (private lodging) November 1-5

A husbandry session Saturday October 30 will, in addition to lectures, discuss guidelines and communication between labs in ZNN/EUFishBioMed.

The ZNN Community meeting 2010, Saturday October 30 will deal with community issues.

The PhD course will include **all** conference lectures and posters, workshop sessions (WS)1-4, journal club sessions (JC)1-4 and, limited to 10 participants, the practical training (LAB)1-5. The theoretical course awards 2ECTS, the full course with theoretical plus practical training 4ECTS.

F29	S30	S31	M01	T02	W03	T04	F05
<i>zebrafish.no phd course 2010</i>							
Thon Hotel Oslo Airport				NVH Campus, Oslo			
	III. Infection model <i>1 lecture</i>	Work- shop ZFN - OPEN protocol	LAB 1	LAB 2	LAB 3	LAB 4	LAB SUM- UP & EVAL
Registration 1030-	IV. Toxicogen-omics						
LUNCH	<i>2 lectures</i>						
I. Omics <i>2 lectures</i>	LUNCH POST/EXHIB	LUNCH	WS 1 forward- reverse genetics & neurotox	WS 2 fish infection models	WS 3 medaka model	WS 4 zebra- fish omics	DEP.
	IV. Cont. <i>1 lecture</i>	DEPART URE					
Coffee/Tea	V. Husbandry <i>1 lecture</i> <i>2 short pres.</i>		Coffee/Tea				
II. Neuro- science <i>2 lectures</i>	POST ER - EXH IB - WINE	ZNN Comm. Meeting	JC 1 forward- reverse genetics & neurotox	JC3 fish infection models	JC 3 medaka model	JC 4 zebra- fish omics	
POSTER EXHIB WINE							
DINNER	Aperitif ZNN2010 DINNER						

October 29, ZNN2010 Conference

1030-1130 Registration

1130-1230 LUNCH

1230 Peter Aleström. Welcome to the ZNN2010 Conference.

1240 Rector Yngvild Wasteson, NVH: Opening address 1.

Research Council Norway: Opening address 2.

1300-1500 SESSION I: Omics

Chair: Ingvild Mikkola.

1300 Ferenc Müller, University of Birmingham: Genomics and functional genomics approaches to decipher enhancer and core promoter interaction specificity in developmental gene transcription (The FUGE-N Lecture).

1400 Peter Aleström, Norwegian School of Veterinary Science: Zebrafish early development-omics.

1500 COFFEE/TEA/FRUITS

1530-1730 SESSION II: Neuroscience

Chair: Max Suster and Pertti Panula

1530 Pertti Panula, University of Helsinki: Modulatory neurotransmitter systems in the zebrafish brain: organization and implications for disease modeling.

1630 Max Suster, Sars Institute, Bergen: Genetic dissection of vertebrate sensory-motor circuits and behavior using zebrafish.

1730-1930 POSTERS, EXHIBITION, WINE & SNACKS

2000 DINNER

October 30, ZNN2010 Conference

0900-1000 SESSION III: Infection model

Chair: Øystein Evensen

0900 Gareth Griffith, University of Oslo: Nanoparticle therapies against tuberculosis in the zebrafish model.

1000-1300 SESSION IV: Toxicogenomics

Chair: Erik Ropstad and Rune Male

1000 Marte Rusten, University of Bergen: Nuclear receptors in zebrafish liver as targets for endocrine disrupting compounds.

1100 *COFFEE/TEA/FRUIT*

1130 Erik Ropstad, Norwegian School of Veterinary Science: Pollutants from the environment affect gene expression in Zebrafish.

ZEBRAFISH NETWORKS

Chair: Peter Alestrom

1230 Uwe Strähle, Karlsruhe Institute of Technology: EUFishBioMed COST BM0804 network - Zebrafish Stock Center, Short Term Scientific Missions (STSMs).
Pertti Panula: Integrative Fish Behavioural Neuroscience Network (Nordforsk 2010-2011).

1300-1400 *LUNCH, POSTER, EXHIBITION*

1400-1500 The EUFishBioMed lecture.

Uwe Strähle, Karlsruhe Institute of Technology: Chaperones, deaminases and other proteins in muscle development and repair.

October 30, ZNN2010 cont'd

1500-1700 SESSION V: HUSBANDRY

Chair: Jan Roger Torp Sørby

- 1500 Tracy S. Peterson, Oregon State University: Overview of Laboratory Zebrafish Pathology - The Expected and Unexpected
- 1600 Hasan Cantas, Norwegian School of Veterinary Science: Cultureable Gut Microbiota Diversity in Norwegian Zebrafish Facilities.
- 1615 Marianne Kraugerud, Norwegian School of Veterinary Science: Natural mixtures of persistent organic pollutants alters follicular dynamics in zebrafish (*Danio rerio*).
- 1630 Zebrafish Guidelines; Communication between labs; Other subjects.

1700-1800 The ZNN Community meeting 2010

PI/PI representatives. Present ZNN Board members: Peter Aleström (Oslo), Ståle Ellingsen (Bergen), Ingvild Mikkola (Tromsø).

1630-1930 POSTERS, EXHIBITION, WINE & SNACKS

- 1945 *ZNN2010 Apéritif*
- 2000 *ZNN2010 Conference DINNER*

October 31, ZNN2010 Zinc Finger Nuclease Workshop

Zinc finger nucleases (ZFNs) are a new class recombinant endonucleases, initially designed for repair of mutations in cancer cells, but more recently developed for basic functional genomics through targeted mutagenesis in the zebrafish germline (and other species). This powerful method that can generate predestinated zebrafish gene knockout mutants is part of the ambitious *Zebrafish Phenome Initiative* and will have great impact on the further development of the zebrafish model (see short review by Stephen Ekker in *Zebrafish* (2008) 5:121-123). **Keith Joung's lab** has developed the **Oligomerized pool engineering (OPEN): an 'open-source' protocol for making customized zinc-finger arrays** (Maeder et al. (2008) *Mol. Cell.* 31: 294-301; Maeder et al. (2009). *Nature Protocols* 4: 1471-1501; see also The Zinc Finger Consortium web site at <http://www.zincfingers.org/scientific-background.htm>). We are happy to have Dr's. Keith Joung and Cherie Ramirez coming to Oslo for sharing his knowledge and experience with customized ZFNs in the ZNN2010 ZFN Workshop.

Further, the ZFN method will be experimentally practiced and demonstrated under supervision of Dr's Joung and Ramirez in the co-organized *zebrafish.no phd course 2010*.

0900-1300 ZNN2010 workshop.

Chair Ståle Ellingsen & Peter Aleström

0900 J.Keith Joung, Massachusetts General Hospital/Harvard Medical School, USA: **Zinc Finger Nucleases in targeted mutagenesis**

1300 LUNCH
DEPARTURE

The **ZNN2010 conference** and the *zebrafish.no phd course 2010* are sponsored through the RCN-FUGE Norwegian Zebrafish Platform Grant No. 183344.

The **ZNN2010 Zinc Finger Nuclease Workshop** and corresponding parts of the PhD course is sponsored through the RCN-HAVBRUK (Aquaculture - An Industry in Growth) Grant No. 176764.

Ferenc Müller

University of Birmingham

Genomics and functional genomics approaches to decipher enhancer and core promoter interaction specificity in developmental gene transcription

F. Müller^{1,2}, Y. Hadzhiev, J. Gehrig,^{1,2} E. Kalmar², N. Li¹, W. M Reischl², U. Liebel², van IJcken³, O. Armant², U Strähle², P. Carninci⁴, E. Stupka⁵, A. Akalin⁶, X. Dong⁶, Y. Sheng⁶, B. Lenhard⁶.

1. University of Birmingham, UK, 2. KIT Karlsruhe, Germany, 3. Erasmus MC, Netherlands, 4. RIKEN Yokohama, Japan, 5, UCL, London, UK, Universitetet I Bergen, Norway

Genome wide studies in mammalian cells revealed an unexpected complexity of transcription initiation mechanisms reflecting differential gene regulation on the core promoter level. Taken together with the apparent variability in the pre-initiation complex composed of members of the TATA binding protein (TBP) family and TBP associated factors (TAFs) it is expected that the core promoter plays a role in responsiveness of genes to short and long range regulation during ontogeny. To study initiation of transcription in animal development, we have globally mapped core promoters in the complexity of the developing embryo. We used zebrafish in which developmental regulation of transcription can be monitored by genomics tools as well as it is a cheap and rapid alternative for in vivo functional assays. We have developed a high throughput automated microscopy tool for the detection of tissue-specific reporter gene activity. We used this technology in tens of thousands of transgenic embryos to map the interaction specificity between enhancers and core promoters in hundreds of combinations. The findings underscore the importance of the core promoter sequence in cis-regulatory interactions. To further explore core promoter diversity in the vertebrate embryo we have carried out a high resolution analysis of the transcriptional landscape by Cap Analysis of Gene Expression (CAGE) during several stages the embryogenesis of zebrafish and identified comprehensively the transcriptional start site usage patterns of genes. Detection of H3K4me3 binding and RNA sequencing helped validating gene and gene promoter predictions. These analyses have uncovered the developmental dynamics of core promoter usage during the maternal to zygotic transition and organogenesis. The CAGE analysis has also revealed an abundance of transcription initiation in exons and introns that are highly dynamic and developmentally regulated.

Peter Aleström

Norwegian School of Veterinary Science

ZEBRAFISH EARLY DEVELOPMENT-OMICS

BasAM, Norwegian School of Veterinary Science and Norwegian Zebrafish Platform, 0033 Oslo, Norway. Peter.Alestrom@nvh.no, <http://zebrafish.no>.

The mechanisms controlling the early stages of zebrafish development are in a genome-wide perspective still not known in great detail. The paradigm is that maternal RNAs prepare for the onset of zygotic gene expression at the midblastula transition (MBT). The mechanisms controlling the fine tuned expression of large number of genes involved in early developmental processes are gradually being characterized at the genomics level¹. In order to further address the dynamic switch from a maternally deposited to a zygotic *de novo* transcribed transcriptome in zebrafish, we have carried out extensive mRNA sequencing and initiated the unraveling of the corresponding epigenetic landscapes of the MBT genome and the changes during the early stages of development².

Using the SOLiD3 platform (Applied Biosystems) for mRNA sequencing, the transcriptomes of 6 early developmental stages (oocyte, 1-, 16/32-, 128/256-cell, MBT and post-MBT) revealed ca 11000 RefSeq genes of which ca 6000 were clustered into 3 main groups according to their differential expression profiles. A large number of alternative splice isomers and novel transcribed regions will contribute to the further annotation of the zebrafish genome. Of the maternally loaded RNAs, a substantial fraction was shown to undergo delayed polyadenylation during the pre-MBT stages, suggesting this being an important mechanism for gene expression control during zebrafish early development.

In order to characterize the dynamics of the zebrafish MBT epigenome, methods for chromatin immunoprecipitation (ChIP), together with DNA methylation (BIS, meDIP) has been established³, recently combined with a 2.1 million probe zebrafish promoter tiling microarray (NimbleGene)⁴. Developmentally-regulated gene promoters (*pou5f1*, *sox2*, *sox3*, *klf4*, *nnr*, *otx1b*, *nes*, *vasa*) of MBT stage embryos all had unmethylated promoter regions but segregated into distinct patterns of chromatin marks at the post translational histone modification (PTM) profile level. Using the high-resolution tiling microarray combined with the mRNA-seq data set, we are presently mapping the correlation between the dynamic genome wide transcriptomes and epigenomes during the early stages of zebrafish development.

¹ Mathavan et al. PLoS Genet 1:260 (2006); Giraldez et al. Science 312:75 (2006); Lindeman et al. 2010. Int. J. Dev. Biol. 54: 803 – 813.

² This work is a collaboration between our lab and the laboratories of Philippe Collas (University of Oslo) and Sinnakaruppan Mathavan (Genome Institute of Singapore).

³ Lindeman et al. Methods Mol. Biol. 567, 75-86 (2009); Wardle et al. Genome Biol 7:R71 (2006); Vastenhouw et al. Nature 464:922 (2010).

⁴ Lindeman et al., submitted.

Pertti Panula

University of Helsinki

Modulatory neurotransmitter systems in the zebrafish brain: organization and implications for disease modeling.

Despite significant structural differences with mammalian brain, the zebrafish brain has the same main regions, neurotransmitters and their receptors as mammals. Functional analysis of neurotransmitter systems is also possible with morpholino oligonucleotides in larval fish, or mutant fish lacking critical enzymes, receptors or transporters. We analyzed numbers of neurons in several important neuron clusters, including the dopaminergic groups, and created 3-dimensional maps of cell groups and fiber projections in larval fish. This allows detailed analysis of consequences of drug treatment, genetic manipulation, or translation inhibition. Combined with quantitative behavioral analysis this approach allows studies on neural basis of behavior and disease processes. Gene duplication has resulted in non-allelic forms of some important genes, like tyrosine hydroxylase (TH). The clusters of neurons expressing TH2 formed four distinct and non-overlapping systems from TH1.

The biochemical and behavioral consequences of either TH1 or TH2 translation inhibition suggested significant contribution to catecholamine synthesis. The histamine-synthesizing enzyme L-histidine decarboxylase was expressed in a single cluster in posterior hypothalamus. Its translation inhibition affected expression of active enzyme for 14 days, as evidenced by histamine HPLC and immunohistochemistry. Thus, the time window of translation inhibition allows at best quick analysis of complex behaviors related to disease mechanisms. Inactivation of disease genes, including PINK1 kinase relevant for Parkinson's disease, affected both TH1 and TH2, and rendered the larvae sensitive to subeffective doses of the neurotoxin MPTP. Larval fish are thus suitable models for studies on basic mechanisms of brain diseases.

Zebrafish offers excellent possibilities to learn about the basic mechanisms important in human diseases.

Supported by Academy of Finland and Sigrid Juselius Foundation.

Maximiliano Suster

Sars International Centre for Marine Molecular Biology

Genetic dissection of vertebrate sensory-motor circuits and behavior using zebrafish.

Essential motor behaviors in vertebrates from swimming in fish to walking in humans are driven by evolutionarily conserved neuronal circuits located in the brain and spinal cord. Given the complexity of the mammalian brain, our understanding of how specific types of neurons and circuits drive and control particular behaviors in vertebrates remains challenging. Our group is developing and using novel genetic approaches in zebrafish to identify and manipulate neuronal circuits that drive and control specialized motor behaviors in vertebrates. Our aim is both to understand how such circuits are assembled and become functional during development, but also to develop therapeutic tools that could contribute to the treatment of motor disorders in humans. Here I will outline how we are combining gene-trapping, BAC transgenesis, fast behavioral imaging and electrophysiology to advance these goals. First, I will present work on the development of genetically-encoded botulinum neurotoxins and their application to studies of basic sensory-motor circuits and behavior. Second, I will describe how we are using Gal4 gene-trapping and BAC transgenesis to identify and manipulate small populations of key locomotor interneurons in the vertebrate brain.

Gareth Griffiths
University of Oslo

Development of nanoparticle-based therapies against pathogenic mycobacteria in the zebrafish model.

Tuberculosis, caused by *Mycobacterium tuberculosis* is now the single most deleterious infectious disease in the world, killing 2 million people annually and latently infecting one-third of the planet. The disease is currently treated with a cocktail of four antibiotics given daily for 6-8 months, a treatment associated with patient non-compliance and multi-drug resistance (MDR). Over the past decade significant improvement in antibiotic therapy has been shown in different animal models by encapsulating the drugs in biodegradable nanoparticles, in most cases made of a polymer of glycolic acid and lactic acid. The key to this system is that the particles can cross epithelial barriers and be naturally taken up via phagocytosis by macrophages, which are also the cells where the intracellular *M.tuberculosis* reside. In these cells the beads are slowly degraded and release their drug content slowly over many days. A consequence of this slow release is that standard daily applications of antibiotics can be replaced by nanoparticle applications every 10-15 days, for example in guinea pig models of tuberculosis. Another advantage is that, since the total systemic concentration of drugs is much lower with the nanoparticle approach than with standard drug treatment this opens the door to testing a wider spectrum of antibiotics that are effective against MDR *M.tuberculosis in vitro*, but only at concentrations that would be toxic when given to humans in the standard, free form (summarized by Griffiths et al, 2010. Nature.Rev. Microbiol, *in press*). We have recently taken advantage of the zebrafish infection system using the fish tuberculosis organism *M.marinum* (GFP) that has been pioneered by the group of Ramakrishnan in Seattle. This group has shown that when these bacteria, that grow best at the same temperature as the fish (28⁰C), are injected into the 30-40h fish embryos they rapidly infect macrophages then form granuloma. In this presentation I will summarize our still preliminary efforts to combine this infection model having green bacteria with the use of fluorescent nanoparticles enclosing antibiotics.

Marte Rusten

University of Bergen

Nuclear receptors in zebrafish liver as targets for endocrine disrupting compounds

RUSTEN M¹, EIDE M¹, MORK A¹, BACHE S¹, DAVIES R¹, LILLE-LANGØY R¹, MALE R¹, GOKSØYR A².

Department of Molecular Biology & ²Department of Biology, University of Bergen, N-5020 Bergen, Norway

Zebrafish has been extensively used in toxicity screening and to identify molecular targets for endocrine disrupting compounds (EDCs) used in EDCs test protocols. Most of the work with EDCs and zebrafish focus on disruption of sexual differentiation and reproduction. However, also other species-specific features in endocrine processes and biological properties need to be considered in establishing such protocols using zebrafish. More knowledge in these matters is also important for the extrapolation of findings in zebrafish to other vertebrate species and in understanding how EDC induced gene expression can lead to disease. The aim of our project is to study a group of ligand activated transcription factors, nuclear receptors (NRs), in zebrafish liver. NR are ligand activated transcription factors that convert extracellular signals into transcriptional responses and in the liver they regulate important biological processes such as glucose homeostasis, metabolism of lipids and steroid hormones and the elimination of potentially toxic compounds from the body. Importantly, NR are affected by EDCs and the aim of our project is to study how EDCs affect NR dependent transcription in zebrafish compared to other species. The gene expression profile of key NRs and target genes have been measured in primary hepatocytes after treatment with control ligand and selected EDCs. We have also evaluated a zebrafish hepatic cell line, ZF-L, as a tool to study NR dependent transcription in this system.

In another part of the project, we are focusing on the nuclear NR1I2 SXR (PXR) system, known to be involved in metabolism and elimination of both xenobiotics and endobiotics. Both exogenous and endogenous chemicals bind SXR and regulate the expression of a large number of enzymes and transporters involved in the organisms' response to the chemical environment. SXR, being activated by an unusually wide range of compounds also exhibit large species differences in ligand specificity. We are performing comparative functional studies of ligand specificity and target gene regulation of NR1I2-like genes from various vertebrate and urochordate species, including Arctic and North Atlantic species such as the polar bear, glaucous gull, Atlantic cod, Atlantic salmon, and *Oikopleura dioica* and compare the activation profile with model organisms such as zebrafish and mice.

The study is supported by the NFR Miljø 2015-program (project number 181888)

Erik Ropstad

Norwegian School of Veterinary Science

Pollutants from the environment affect gene expression in Zebrafish.

Erik Ropstad¹, Irena M. Grześ⁵, Rasoul Nourizadeh-Lillabadi^{2*}, Camilla Almaas¹, Vidar Berg³, Mona Aleksandersen², Janneche Utne Skåre^{3,4}, Peter Alestrøm², Jan L. Lyche¹

¹ Dept. Production Animal Clinical Science, Norwegian School of Veterinary Science, POB 8146 Dep., N-0033 Oslo, Norway

² Dept. of Basic Sciences and Aquatic Medicine, Norwegian School of Veterinary Science, POB 8146 Dep., N-0033 Oslo, Norway.

³ Dept. Food safety and Infection Biology, Norwegian School of Veterinary Science, POB 8146 Dep., N-0033 Oslo, Norway.

⁴ National Veterinary Institute, POB 8156 Dep., N-0033 Oslo, Norway.

⁵ Jagellonian University, Krakow, Poland

Effects of real life mixtures of persistent organic pollutants (POPs) harvested from aquatic ecosystems in Norway were studied in an *in vivo* zebrafish model. POPs were extracted from burbot (*Lota lota*) liver from two lakes, Lake Losna and Lake Mjøsa, and exposed to zebrafish through the diet in a two-generation study. Effects on survival, growth, sex ratio, timing of puberty were investigated. In addition, the biomarkers 7-ethoxyresorufin-*O*-deethylase (EROD) and vitellogenin (VTG) were measured.

The ratio of contaminant levels in extracts collected from Lake Mjøsa/Lake Losna were 6, 10, and 270 for polychlorinated biphenyls (PCB), dichlorodiphenyltrichloroethanes (DDT) and polybrominated diphenylethers (PBDE) respectively. The concentration range of POP measured in zebrafish was lower than in burbot originating from Lake Mjøsa, but comparable to concentrations previously reported in humans and wildlife.

Phenotypic effects observed in both exposure groups included earlier onset of puberty, increased male/female sex ratio, and differences in body weight at 5 mo of age. Interestingly, genome-wide transcription profiling identified functional networks of genes, in which key regulators of weight homeostasis, steroid hormone functions and insulin signaling occupied central positions. Parental exposure effects on the offspring was indicated by demonstration of lower egg production from exposed females and increased mortality in early embryos originating from exposed parents. The results indicated that transcriptomics changes included genes related to embryonic development, including cell proliferation and apoptosis, cell migration, molecular transport, and organ development. Effects caused by low, background exposure (Lake Losna) were as pronounced or even more severe than effects caused by high concentration exposure (Lake Mjøsa).

Uwe Strähle

Karlsruhe Institute of Technology

Chaperones, deaminases and other proteins in muscle development and repair.

The chaperones Unc45b and Hsp90a are essential for folding of myosin from worms to men. We show that zebrafish Unc45b, but not Hsp90a, binds to the putative cytidine deaminase Apobec2 (Apo2) in an interaction that requires the UCS and central domains of Unc45b. Morpholino oligonucleotide-mediated knock-down Apo2a and Apo2b causes a dystrophic phenotype in the zebrafish skeletal musculature and impairs heart function. These phenotypic traits are shared with mutants of *unc45b*, but not with *hsp90a* mutants. Apo2a and -2b act non-redundantly and bind to each other *in vitro*, suggesting a heteromeric functional complex. Our results demonstrate that Unc45b and Apo2 proteins act in a Hsp90a-independent pathway that is required for integrity of the myosepta and myofibre attachment. Since the only known function of Unc45b is that of a chaperone, Apo2 proteins may be clients of Unc45b but other yet unidentified processes cannot be excluded.

Tracy S. Peterson

Oregon State University

Overview of Laboratory Zebrafish Pathology - The Expected and Unexpected

The use of zebrafish as laboratory animals over the past decade has increased significantly. Understanding of zebrafish infectious diseases, non-infectious processes and neoplasia initially lagged behind those of other lab animals but today our knowledge of these diseases has progressed and enabled the creation of specific pathogen-free (SPF) zebrafish lines. This overview of zebrafish pathology, although non-comprehensive, is intended to provide a sufficient understanding of the more common and expected diseases as well as some of the unexpected diseases that occur in the routine health management of zebrafish colonies. Infectious diseases that will be covered include mycobacteriosis, microsporidiosis, Gram-negative bacterial infections, enteric nematodiasis (*Pseudocapillaria tomentosa*) and opportunistic fungal infections. Non-infectious processes will also be discussed, including those that are truly incidental in nature such as nephrocalcinosis, gas bubble disease, senescent changes, congenital dermomelanosis and nutritional diseases. Spontaneous neoplastic diseases can occur in zebrafish, particularly in fish greater than 1.5 to 2 years of age. Spermatocytic seminoma, ultimobranchial adenoma/carcinoma and hepatocellular adenoma tend to be the more commonly seen neoplastic diseases in zebrafish colonies. Less common neoplastic diseases, some of which may be husbandry-related, include intestinal adenocarcinoma, small cell carcinoma, pancreatic carcinoma, nephroblastoma, malignant peripheral nerve sheath tumor, neuroblastoma and hemangiosarcoma.

Hasan Cantas

Norwegian School of Veterinary Science

Culture-able Gut Microbiota Diversity in Norwegian Zebrafish Facilities

H. Cantas^{1†}, J.R. Torp², P. Alestrom², H. Sorum¹

¹ Department of Food Safety and Infection Biology, Norwegian School of Veterinary Science, Oslo/Norway

² Department of Basic Sciences and Aquatic Medicine, Norwegian School of Veterinary Science, Oslo/Norway

†Corresponding author. Tel.: + 47-40683725 E-mail address: Hasan.Cantas@nvh.no (H. Cantas)

Background: The gastrointestinal tract of fish consists of a very complex and dynamic microbial ecosystem. To the best of our knowledge the normal cultureable zebrafish intestinal microbiota and the impact of various environmental factors on its composition has remained not well defined. Therefore the aim of this study was to compare the composition of the cultureable part of the intestinal microbiota of TAB strain Zebrafish stocked in different facilities. We evaluated the effect on the gut composition of the cultureable gut microbiota of i) the status of sexual maturity and gender as well as the impact of ii) husbandry related external factors like UV-light exposure of re-circulated water and the bacterial contents of tank water and of the feed.

Materials and methods: The study was an open and single central trial with a stratified, sequential parallel group statistical design in four Zebrafish Network Norway member Labs (Aleström Lab, NIFES Lab, UIB Lab, SARS Lab). A total of seventy-two TAB line Zebrafish were used as a reference population. Traditional bacterial culturing methods, morphological observation, biochemical characterization using commercial kits and 16s rDNA sequencing techniques were performed in order to characterize the bacterial isolates of the healthy zebrafish intestinal tract, water and feed. Also two identified Gram negative colonies per sample were selected for phenotypical antibiotic resistance testing.

Results: A total of 13 morphologically different bacterial species were the most prevalent: *Aeromonas hydrophila*, *A. sobria*, *Vibrio parahemolyticus*, *Photobacterium damsela*, *Pseudomonas aeruginosa*, *P. fluorescens*, *P. luteola*, *Commamonas testesteroni*, *Ochrabacterium antropi*, *Staphylococcus cohnii*, *S. epidermidis*, *S. capitis* and *S. warnerii*. The number of each bacterium was associated with different sampling points. At facility level Aleström Lab was selected as a baseline that was compared with the others. Herein only NIFES had significantly higher levels of total bacterial growth (OR=2.025; 95% CI=1.73-2.38) whereas from the UIB Lab (OR=1.008; 95% CI=0.86-1.18) and the SARS Lab (OR=1.122; 95% CI=0.89-1.41) it was found similar figures

when compared with each other. At fish level, the sexual immature individuals had a significantly higher level of harvested total bacterial growth than the mature ones (OR=0.823; 95% CI=0.73-0.93). Also the posterior intestinal segment demonstrated a higher degree of cultureable bacterial contents than the anterior segment (OR=4.998; 95% CI=4.38-5.71). On the other hand no statistically significant differences were found between the male and female fish (OR=1.005; 95% CI=0.89-1.13) with regards to total bacterial growth, based on an ordered logistic regression model and chi square test.

Conclusion: In the first comprehensive investigation of microbiota of healthy TAB strain zebrafish we could identify significant i) diversities among facilities, ii) higher degree of bacteria growth at sexually immature fish and posterior intestinal segment iii) similarity between gender. Promising statistical significant differences and similarities revealed by culture based isolation techniques need to be verified by further culture independent analysis. Also our study emphasized a link between management and the intestinal gut microbiota balance. This connection supports that optimal management prevents opportunistic infections.

Keywords: TAB line Zebrafish, Gut Microbiota, 20 Api NE, 16s rDNA PCR

Marianne Kraugerud

Norwegian School of Veterinary Science

Effects of natural mixtures of persistent organic pollutants on ovarian follicles in zebrafish (*Danio rerio*).

Marianne Kraugerud, Richard William Doughty, Mona Aleksandersen, Nina Hårdnes, Camilla Karlsson, Vidar Berg, Jan Lyche, Janneche Utne Skaare, Peter Aleström, Erik Ropstad

Assessment of exposure to mixtures of pollutants is a prerequisite for evaluating the impact of xenobiotics on the ecosystem. In the present study, pollutants were extracted from livers of burbot (*Lota lota*) from two freshwater systems in Norway. Zebrafish (*Danio rerio*) were exposed through food from 6 days post fertilization until their sacrifice at onset of sexual maturity (5 months of age). Exposure groups included two concentrations of extract from lake Mjøsa ("Mjøsa low" and "Mjøsa high"), containing high levels of polybrominated biphenyl ethers, polychlorinated biphenyls and dichlorodiphenyltrichloroethane metabolites and a single concentration of extract from lake Losna ("Losna"), containing background levels of these compounds. One final group served as control.

Concentrations of pollutants detected in the exposed zebrafish were lower than concentrations detected in burbot inhabiting Lake Mjøsa and Lake Losna, but comparable to background exposure of humans and wildlife. Fulton's condition factor and body length was not statistically different between the exposure groups, however fish within the "Losna" group had significantly higher body weights. Ovarian staging carried out semiquantitatively demonstrated a significantly greater relative proportion of perinucleolar oocytes in fish exposed to "Losna" and "Mjøsa high" and a significantly greater relative numbers of cortical alveolar oocytes in fish exposed to "Mjøsa high". The relative proportion of late vitellogenic oocytes were significantly lower in the "Losna" and the "Mjøsa high" exposure groups. Immunohistochemistry against the proliferative marker PCNA detected a significant decrease in proliferation in the liver hepatocytes and ovarian granulosa cells of fish exposed to "Mjøsa low" and "Mjøsa high". These findings suggest that exposure to real-life mixtures of pollutants affects follicle dynamics in the ovary and proliferation in the liver.

J. Keith Joung

Massachusetts General Hospital & Harvard Medical School

Zinc Finger Nucleases in targeted mutagenesis

Engineered zinc-finger nucleases (ZFNs) form the basis of a broadly applicable method for targeted, efficient modification of eukaryotic genomes including zebrafish. In previous work, we described OPEN (Oligomerized Pool ENgineering), an 'open-source,' combinatorial selection-based method for engineering zinc-finger arrays that function well as ZFNs. We and our collaborators have shown that OPEN ZFNs can induce mutations with high efficiency in zebrafish, plants, and somatic and pluripotent human cells. OPEN selections are carried out in *Escherichia coli* using a bacterial two-hybrid system and do not require specialized equipment. Using OPEN, researchers can generate multiple, customized ZFNs in 8 weeks or less. In this workshop, we will provide an overview of the method as well as practical tips and suggestions for carrying out OPEN selections. Recent updates and improvements to the method will also be described.

P 1:

Characterization of medaka LH-producing gonadotrope cells

E. Ager-Wick*, R.A.U. Strandabø*, F-A. Weltzien and T.M. Haug

*Ager-Wick and Strandabø contributed equally to this poster

Puberty and sexual maturation in vertebrates are regulated through increased activity in the brain-pituitary-gonad axis (BPG). Our work focuses on the pituitary, specifically on gonadotrope cells. The Weltzien-Haug group has developed a transgenic line of medaka (*Oryzias latipes*) that has GFP coupled to the promoter of luteinizing hormone beta subunit (LH β), which allows for selection of LH-producing cells. Despite the small size of the medaka pituitary we have developed a method for dissecting and dissociating the pituitaries. The dispersed cells are made into primary cell cultures, where LH-producing gonadotrope cells can easily be identified by the expression of GFP.

These cells are used for two main purposes: electrophysiological characterization and global gene expression analysis by high throughput sequencing (HTS). The electrophysiological studies are combined with calcium-imaging to elucidate the response to gonadotropin-releasing hormone (GnRH) in LH-producing cells. Spontaneous action potential activity in pituitary cells has been observed, and the intracellular calcium response to different forms of GnRH is in the process of being investigated.

For global gene expression analysis, GFP-positive cells are sorted by FACS (Fluorescence Activated Cell Sorting) from the total suspension of dissociated cells. The limited amount of LH-producing gonadotropes in each pituitary is a challenge, due to the amount of RNA needed for HTS. A pilot experiment based on 220 ng of total RNA extracted from 100 medaka pituitaries was done on the Illumina GAII platform to test whether this amount of material is sufficient for sequencing without further pre-amplification. The quality of the sequencing result shows that despite the small amount of starting material, we succeeded in sequencing the transcriptome of the LH-producing gonadotropes, which allows us to study gene expression in these particular cells.

These studies will help us to gain a deeper understanding of the regulation of LH-producing cells in medaka during puberty.

P2:

Tagging of zebrafish (*Danio rerio*) for behavioural studies

Houner Ismail, Josefin Dahlbom, Svante Winberg
Dept. of Neuroscience, Uppsala University, Sweden

In behavioural studies, it is often important to distinguish between two or more individuals. In zebrafish, this can be difficult since they are fast-moving and look very much alike. It is therefore important to have a way to tag the fish that can be used in experiments that last several days. One usual way of marking other fish species is fin clipping. This is feasible on zebrafish as well, but due to rapid regeneration of the fins it is not a suitable marking method for more long-term studies. Another common method is tagging. However, even the smallest commercial tags available are too large to be used on zebrafish. Often zebrafish are tagged subcutaneous dye injections but these require UV-light for visualization.

We have developed a tagging technique for zebrafish that is visible to the naked eye and does not affect the normal behaviour of the fish. A nylon monofilament (fishing line) is pulled through the dorsal muscle ventral to the dorsal fin. The ends are burned and painted with nail-polish in different colour combinations.

In this experiment we examined the effect of this tagging technique on whole-body levels of cortisol, and if gender had any effect on this stress response. Fish were sampled at 1 h, 5h and five days after tagging.

Cortisol will be analysed using a validated Radioimmuno assay.

P3:

Gal4- UAS lines as tools for zebrafish research

Puja Gupta, Anders Fjose

Background: The Gal4 is a transcriptional activator from yeast that activates transcription by binding to a UAS (Upstream Activating Sequence) element. Initially adapted from the yeast to be used in drosophila, it has recently been established as a tool in zebrafish (*Danio rerio*). Transgenic zebrafish lines expressing Gal4 in a cell-type specific manner can routinely be generated using a vector that integrates into the genome and expresses Gal4 in response to activation of a minimal core promoter in the vector by an endogenous enhancer (enhancer trapping). Ectopic expression of selected genes can then be achieved by using a second UAS transgene, which would be trans-activated by Gal4 in the same cells that express Gal4.

Method: We have performed an enhancer trap screen using the Tol2 vector SAGVG that contains a modified Gal4 sequence (Gal4-VP16) under a minimal promoter, followed by a UAS-GFP cassette (Davison et al. [2007] *Dev. Biol.* 304: 811-824). Screening F1 embryos for EGFP expression, we obtained ~100 transgenic lines with restricted GFP expression. We crossed selected enhancertrap lines against a transgenic line containing a UAS-RFP construct, to positively identify Gal4 transactivation.

Results: We have characterized 16 zebrafish lines with cell specific expression in blood cells, endothelial cells, specific retinal layers, muscle somites or rhombomeres. Of these, 11 lines express Gal4 able to transactivate a UAS-RFP construct. The other 5 lines have GFP expressed under the direct control of an endogenous enhancer not involving Gal4 expression. We have successfully mapped the genomic location of the integrated vector for 10 of the transgenic lines. For 2 of the mapped lines, we have determined a nearby gene with similar/identical expression, indicating the identity of the activating, endogenous enhancer. We have also observed mosaicism in expression of reporter genes between cis-activated (EGFP) and trans-activated (RFP) UAS transgenes.

P4:

Characterization of medaka pituitary gonadotrope cells using a stable LH transgenic fish line

Hildahl J, Sandvik GS, Lifjeld R, Nagahama Y, Haug TM, Okubo K, Weltzien FA

Japanese medaka (*Oryzia latipes*) is, similar to zebrafish, a powerful model for functional genomic and systems biology research. It shares many of the advantages of the zebrafish model such as a sequenced genome, rapid development in transparent eggs, short generation time and a wealth of advanced biotechnological tools. A few advantages of the medaka model are a greater temperature and salinity tolerance for experimental manipulation, closer phylogenetic relationship to many important aquaculture species, and a well-defined sexual development and reproductive physiology. For the latter reason we have chosen to use the medaka model for our research to characterize the pituitary gonadotrope cells that produce and secrete luteinizing hormone (LH) and follicle-stimulating hormone (FSH). To this aim we have developed a stable transgenic line of medaka with the LH beta gene (*lhβ*) promoter driving GFP expression by microinjection of a BAC construct with hrGFP inserted downstream of the LH promoter. This line has been used to trace the development of LH gonadotropes using confocal microscopy. We demonstrate that *lhβ* is expressed early during development of the pituitary, already after 30 hpf. Expression is initially more dispersed and extends anteriorly in the developing brain and later becomes more consolidated in the primordial pituitary. These data lay the framework for future research into the function of LH during early development and pituitary developmental studies. This transgenic line also provides the technological basis for further characterization of gonadotropes as seen in a second poster.

P5:

Non-animal (alternative) testing methods for REACH (alterREACH)

Maria Hultman^{1,2}, Peter Aleström³, Mark Cronin⁴, Øystein Evensen³, Katherine Langford¹, Adam Lillicrap¹, Kenneth Macrae¹, Bjørn Olav Rosseland², Kristin Schirmer⁶, Stefan Scholz⁵, Kevin V. Thomas¹, Knut Erik Tollefsen^{1,2}.

¹ Norwegian Institute for Water Research (NIVA), Oslo, Norway.

² University of Life Sciences (UMB), Ås, Norway.

³ Norwegian School of Veterinary Science (NVH), Oslo, Norway.

⁴ Liverpool John Moores University (LJMU), United Kingdom.

⁵ Helmholtz Centre for Environmental Research (UFZ), Leipzig, Germany.

⁶ Swiss Federal Institute of Aquatic Science and Technology (EAWAG),

Dübendorf, Switzerland

The **Registration, Evaluation, Authorisation and Restriction of Chemical Substances (REACH)** is the European chemical regulatory legislation for the new and existing chemical substances. The new REACH directives require that chemicals believed to be persistent, bioaccumulative and/or toxic (PBT) have to undergo regulatory testing using aquatic vertebrates. Current estimates are that about 30,000 single chemicals may require testing with up to three million fish using currently available and validated methods. With the strong drive towards implementing the 3 R's (reduction, refinement and replacement) into ecotoxicological testing, need for developing and evaluating alternative (non-animal) experimental methods is clearly warranted. This multi-disciplinary project, involving various international research groups, intends to meet this challenge through the development and evaluation of alternative test methods to carry out rapid, reproducible screening for the bioaccumulative and toxic properties of chemicals. Ecotoxicological testing using zebrafish (*Danio rerio*) embryos, complimented by *in silico* quantitative structural activity relationship (QSAR) models, *in vitro* methods (cell cultures) and toxicogenomics, will be assessed. Results will then be compared to findings from *in vivo* experiments to evaluate whether these approaches may be applied as part of the regulatory testing within REACH.

P6:

Incidence of spontaneous microscopic lesions in aged zebrafish: A cross-sectional study.

Marianne Kraugerud, Richard William Doughty, Jan Roger Torp, Peter Aleström,

The zebrafish (*Danio rerio*) has emerged as a leading animal model for biomedical research. There is a growing need for pathology support in the use of zebrafish for basic biomedical research, translational research, colony health screening and tumor pathology. To optimize the use of pathology in zebrafish studies, a thorough knowledge of spontaneous and age-related lesions of the various strains of zebrafish is required.

The purpose of this study was to determine the incidence, type, and severity of spontaneous findings in aged, wild-type, local pet store derived zebrafish from the Alestrom's Zebrafish Lab, Norwegian School of Veterinary Science, Oslo. All fish were retired brood stock and > 18 months of age. A total of 97 fish were examined including 39 males and 58 females. After euthanasia, the fish were fixed in formalin, and sectioned longitudinally along the midline. The fish were processed, sectioned and stained with haematoxylin and eosin. In all fish, at least one spontaneous lesion was identified.

In the male fish, seminoma was the most commonly recorded finding (41%). Other lesions identified were biliary hyperplasia and fibrosis (38%), multifocal granulomatous inflammation (13%), cholangioma and cholangiocarcinoma (8%), pancreatic adenocarcinoma (5%), a hepatocellular adenoma (3%), one leukaemia/lymphoma (3%) and one ultimobranchial gland tumour (3%).

In the female fish, egg-associated inflammation was the most common finding, being observed in 48 % of fish. Typically this lesion was associated with fibrosis and extensive cholesterol cleft formation. Biliary hyperplasia and fibrosis was present with an incidence of 41%. Other lesions observed include multifocal granulomatous inflammation (22%), dysgerminoma (10%), cholangioma and cholangiocarcinoma (7%), hepatocellular adenoma and foci of cellular alteration (7%), a spindle cell sarcoma (2%), a pancreatic adenocarcinoma (2%) and a renal cell adenoma (2%).

These data show that aged zebrafish show a range of spontaneous lesions, and highlights the need to develop baseline data on this topic. Further studies are required looking at zebrafish at various ages are required.

P7:

Knockdown of the vertebrate $\alpha 4a$ glycine receptor subunit causes hyperplexia-related tactile-evoked locomotor defects in zebrafish

Kreneisz O.¹, James V.², Kawakami K.³, Harvey R.J.² and Suster M.L.¹

¹ Sars International Centre for Marine Molecular Biology, Bergen, Norway.

² Department of Pharmacology, The School of Pharmacy, London, UK.

³ Division of Molecular and Developmental Biology, National Institute of Genetics, Mishima, Shizuoka, Japan

Survival in nature depends crucially on a controlled response to a wide range of sensory stimuli. Humans who develop hyperplexia (startle disease) show an exaggerated startle response characterised by muscle stiffness in response to acoustic or tactile stimuli, resulting in neonatal apnea episodes. This condition is caused by defects in postsynaptic glycine receptors (GlyRs) or presynaptic glycine transporters that compromise inhibitory synaptic transmission in the central nervous system. Here we show that knockdown of the glycine receptor $\alpha 4a$ subunit in zebrafish (GlyR $\alpha 4a$) with a splice-site morpholino results in an exaggerated startle response and abnormal touch-evoked escape behavior. Application of tactile stimuli to the head, tail or yolk in $\alpha 4a$ morphant larvae triggers an incomplete C-bend, followed by strong but transient bilateral cramping of the upper trunk for several seconds. This hyperplexia-like phenotype appears as early as 35 hpf and can be fully rescued by expression of exogenous GlyR $\alpha 4a$ subunit mRNA. To understand the contribution of the $\alpha 4a$ subunit to startle-related phenotypes and the neuronal circuits involved in this response, we are taking advantage of a Gal4 gene-trap insertion in the *glra4a* locus. When this Gal4 line is mated with a UAS:GFP reporter line, double transgenic progeny exhibit GFP expression in a small subset of commissural interneurons in the hindbrain and spinal cord. We are using this transgenic line to characterise the role of GlyR $\alpha 4a$ within the neuronal circuits controlling escape behaviour through a combination of targeted single-cell PCR, patch-clamp recordings from GFP-labeled neurons and dual neuronal and muscle recordings.

P8:

Tiling Histone H3 Lysine Methylation in Zebrafish Using High-density Microarrays

Leif C. Lindeman¹, Andrew H. Reiner¹, Sinnakaruppan Mathavan², Håvard Aanes³, Peter Aleström³ and Philippe Collas¹

¹Institute of Basic Medical Sciences, Faculty of Medicine, University of Oslo, and Norwegian Center for Stem Cell Research, 0317 Oslo, Norway, ²Stem Cell and Developmental biology, Genome Institute of Singapore, Biopolis, Singapore, ³BasAM, Norwegian School of Veterinary Science, Oslo, Norway.

Uncovering epigenetic states by chromatin immunoprecipitation (ChIP) and microarray hybridization has largely contributed to the understanding of gene regulation at the genome-scale level. Many studies have been carried out in mice and humans, however only limited information exists to date for non-mammalian vertebrate species, particularly zebrafish (1-3), and in a developmental context.

We report here a 2.1 million feature high-resolution tiling microarray for ChIP-on-chip interrogations of epigenetic states in the zebrafish (*Danio rerio*) genome. The array covers 251 megabases of the genome at 92 base-pair resolution, including ~15 kb of all RefSeq promoters and >5 kb in the 5' end of coding regions. We identify with high reproducibility in an embryo-derived cell line, genomic regions enriched in H3K4me3, H3K27me3 or co-enriched in both modifications. These regions are associated with distinct transcriptional status and with genes linked to distinct functional categories. During early embryo development, sequential enrichment of distinct epigenetic marks takes place on a genome-scale level, with the earliest mark detected prior to zygotic gene activation.

We have designed for the scientific community a comprehensive tiling microarray for investigations of epigenetic states in zebrafish, a widely used developmental and disease model organism.

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P9:

Erik-Jan Lock

Toxaphene replaced DDT as the world's most heavily used pesticide in the early 1970s. It is distinctively bioaccumulated in aquatic biota and considered to be a persistent organic pollutant by the Stockholm convention and banned worldwide since 2004. Technical toxaphene mixtures show a complex composition of more than 200 identified compounds of mostly polychlorinated bornanes. Three chlorobornanes (CHBs) are present in marine fish in relatively high concentrations; CHB 26, 50 and 62 (Σ highCHB), and can compose the majority of the toxaphene response in biota. It is therefore presumed that these three indicator congeners will be dominant in regard to human toxaphene uptake, especially with respect to European fishery and aquaculture products. Transformation of these CHBs in soil or in sediment occurs slowly under anaerobic conditions, mostly by means of reductive dechlorination. The resulting residue patterns are shifted toward lower-chlorinated compounds when compared to the technical mixture. There is evidence that toxaphene becomes more toxic to the environment after transformation to lower chlorinated compounds. A decrease in the total amount of toxaphene during environmental breakdown would then be compensated for, at least in part, by the higher toxicity of weathered toxaphene. The aim of the study presented here is to compare dietary exposure to lower chlorinated CHBs 40, 41, 42a and 44 (Σ lowCHB) with dietary exposure to Σ highCHB. Six week old zebrafish were fed 40 days with a commercial diet contaminated with either Σ highCHB, Σ lowCHB or both in two different concentrations. The first concentration is the current upper limit for toxaphene in fish feed, 0.05 mg/kg, the second concentration is ten times higher (0.5 mg/kg). Accumulation of the individual congeners into muscle tissue was measured as well as gene expression of phase I and phase II enzymes in liver.

P10:

Anti-Müllerian hormone overexpression results in a reduced incidence of expression of the oocyte marker *HIM* in zebrafish juveniles.

Katrine S. Skaar¹, Lisbeth C. Olsen^{1,2}, Jan Bogerd³, Knut H. Jensen⁴, Rüdiger W. Schulz³, Rune Male¹

¹Department of Molecular Biology and ⁴Department of Biology, University of Bergen.

²Sars International Centre for Marine Molecular Biology, Bergen.

³Department of Biology, University of Utrecht, The Netherlands.

Sex chromosomes have not been identified in zebrafish and the mechanism of sex determination is poorly understood. Several candidate genes for sex determination and sex differentiation have been characterised in zebrafish. Sex differences in gene expression of these autosomal genes may play a role in controlling sex differentiation. The current view is that zebrafish relies on a multigenic sex determination system, which can be influenced by environmental factors.

In this study, we have addressed whether over-expression of Anti-Müllerian hormone (Amh) during gonadal differentiation in zebrafish could influence sex differentiation. Amh is a member of the transforming growth factor β (TGF- β) superfamily and participates both in the regulation of germ cell proliferation and sex differentiation in medaka.

Somatic and germline markers were monitored in wild-type juvenile zebrafish at 28 and 33 days post fertilisation by reverse transcriptase PCR (RT-PCR). The linker histone *HIM* was followed by *in situ* hybridisation and established as a primordial germ cell marker. *HIM* expression was restricted to oocytes as shown by coexpression with different oocyte markers like the *vas-l* splice variant, the transcription factor *figa* and the granulosa cell marker *cyp19a1a*. A transgenic zebrafish line (*hsp:amh*) was developed to induce *amh* overexpression by heat treatment during gonadal differentiation. We observed a significant reduction in the number of individuals expressing *HIM* in the juveniles with heat-induced *amh* overexpression compared with control groups. Statistical analyses showed that this reduction was linked to over-expression of the *hsp:amh* transgene and not the heat treatment itself. The reduced level of *HIM* in heat-treated transgenic juveniles indicates a reduced number of oocytes compared with non-treated transgenic fish.

P11:

Haraldur Thorsteinsson

Zebrafish (*Danio rerio*) have recently been established as a model in sleep research. Sleep patterns, however, differ widely within species across development. To date sleep studies have been performed in larval and adult zebrafish but no efforts have been made to document the ontogeny of sleep-wake cycles. In the current experiment we, therefore, use an automated behavioral recording system to measure sleep-wake cycles in zebrafish and, for the first time, describe their sleep-wake cycle development across ontogeny; the data are contrasted with human data.

In adult humans the duration of sleep bouts exhibits an exponential distribution with the rule $P(t) \sim \exp(-t/\tau)$ where t is an individual sleep bout, whereas, wake bouts exhibit a power-law distribution with the rule $P(t) \sim t^{-\alpha}$ where t is an individual wake bout. The wake bouts exhibit a scale-free power law behavior with an exponent, α , that remains constant across mammalian species (humans, cats, rats, and mice). In contrast, sleep bout durations follow an exponential distribution where τ represents a characteristic time scale whose main determinants are body size and metabolic rate. In neonates (rats) both sleep and wake bouts exhibit exponential distribution immediately after birth, with a clear power-law behavior of wake bouts emerging only after the second postnatal week; this occurs in spite of very little change in the overall duration of wake bouts; τ , on the other hand, increases with age.

SPECIFIC AIM: To describe the ontogeny of sleep-wake cycles in zebrafish and contrast the data with the corresponding mammalian data.

P12:

Hepatic transcriptional responses in Zebrafish (*Danio rerio*) exposed to waterborne Diclofenac.

Knut-Erik Tollefsen^{1,2}, Tor Fredrik Holth^{1,3}, Kathrine Langford¹, Kevin V. Thomas.

¹Norwegian Institute for Water Research (NIVA), Oslo, Norway; ²University of Life Sciences (UMB), Ås, Norway, ³University of Oslo, Oslo, Norway.

Diclofenac is a non-steroidal anti-inflammatory drug (NSAID) used to reduce inflammation and pain (analgesic) by patients suffering from arthritis or ankylosing spondylitis. The exact mechanism of action of diclofenac is not entirely known, but it is thought to inhibit prostaglandin synthesis by inhibition of cyclooxygenase (COX), reduce leukotriene production by inhibiting the lipoxygenase pathways, block voltage-dependent sodium channels and block acid-sensing ion channels to achieve pain relief. Although diclofenac is considered benign for human and veterinary medicine, this pharmaceutical has caused acute kidney failure and a drastic 99% decline in vultures on the Indian subcontinent. Sub-lethal and non-target effects of diclofenac on aquatic organisms is poorly reported, thus warranting in-depth studies on potential ecological side effects of the use of human and veterinary pharmaceuticals. The current study presents the hepatic transcriptional response in Zebrafish (*Danio rerio*) after 48h waterborne exposure to 1-1000 ug/L Diclofenac using a 44k Zebrafish oligomicroarray as a pre-screening tool for identifying potential side effects of the drug. As many as 681 putative genes were differentially expressed between control and exposed groups, whereof 364 were down-regulated, 66 displayed low-dose induction and 251 displayed traditional concentration-dependent induction. Although the transcriptional response where considerable less pronounced than that for other pharmaceuticals such as the oral contraceptive estrogen 17 α -ethynylestradiol, several key genes were identified of being toxicologically relevant. Potential side-effects will be discussed in light of toxicological pathways being overrepresented in the exposed groups.

P13:

Sucralose in the environment - is there a reason for concern for wildlife effects?

Knut Erik Tollefsen, Adam Lillicrap, Katherine Langford, Kevin Thomas

Norwegian Institute for Water Research (NIVA), Oslo, Norway

Intense (artificial) sweeteners are produced and used in large quantities in all major markets of the world as low calorie sugar substitutes and in some cases, such as sucralose, also as additives in cosmetics. Many of these sweeteners pass through the body either unaltered or as modified compounds and are ultimately released to the environment predominantly through the sewage system. Some of these sweeteners have been detected in various environmental compartments and has raised concern about potential biological effects in non-target species living in areas receiving discharges from anthropological activities. Existing data on fate and effects of intense sweeteners in the environment and novel experimental data for the accumulation and genomic (transcriptional) effects of the intense sweetener sucralose will be presented to elucidate whether these compounds may cause adverse effects to aquatic animals such as algae, crustaceans and fish. The bioaccumulation studies, which were performed with the algae *Pseudokirchneriella subcapitata*, the crustacean *Daphnia magna* and zebrafish (*Danio rerio*), were all conducted as semi-static exposure studies over a period of 48-72 days with two concentrations of sucralose (10 and 100 mg/L), with analytical determination of sucralose in both water and biota. Additionally, the hepatic transcriptional response in Zebrafish (*Danio rerio*) after 48h waterborne exposure to 1-100 mg/L sucralose was determined by microarray-assisted analysis as a pre-screening tool for identifying potential biological effects of the compound in non-target organisms. A combination of data from literature and the experimental approaches will be used to assess the risk posed to aquatic organism under relevant exposure scenarios.

P14:

HMGB1 (amphoterin) is required for zebrafish forebrain development

Xiang Zhao,¹ Juha Kuja-Panula,¹ Ari Rouhiainen,¹ Pertti Panula,^{1,2}
and Heikki Rauvala^{1,3}

¹ Neuroscience Center, University of Helsinki, Finland; ² Institute of Biomedicine/Anatomy, University of Helsinki, Finland

Hmgb1 (*high mobility group B1; amphoterin*) is highly expressed in brain during early development of vertebrate and nonvertebrate species. However, its role in brain development remains elusive. Here we have cloned the zebrafish *Hmgb1* and designed a method to specifically manipulate *Hmgb1* expression using injection of morpholino oligonucleotides (MOs) or *Hmgb1* mRNA. The HMGB1 knockdown morphants produced by injection of three different MOs display a characteristic phenotype with smaller size, smaller brain width and shorter distance between the eyes (over 80 % in larvae in which HMGB1 translation is inhibited; $p < 0.05$ compared to noninjected, mispaired MO injected or mRNA rescued larvae; 200 larvae examined in each group). Closer examination of the phenotype reveals severe defects in the development of the forebrain that lacks catecholaminergic networks in anterior diencephalon and in telencephalon. The HMGB1 morphant is deficient in survival and proliferation of neural progenitors and displays aberrant Wnt8 signalling. The mechanism of HMGB1-dependent progenitor survival involves the neuronal transmembrane protein AMIGO (**amphoterin-induced gene and orf**) 1, the expression of which is regulated by HMGB1 *in vivo*. Our data demonstrate that HMGB1 is a critical factor for brain development, enabling survival and proliferation of neural progenitors that will form the forebrain structures.

Last name	First name	E-mail	Institution/Organization
Ager-Wick	Eirill	eirill.ager-wick@nvh.no	Norges Veterinærhøgskole
Aleksandersen	Mona	mona.aleksandersen@nvh.no	Norwegian School of Veterinary Science
Aleström	Peter	peter.alestrom@nvh.no	Norwegian School of Veterinary Science
Amlie	Kari Elisabeth	kari.amlie@sial.com	Sigma-Aldrich
Berg	Vidar	vidar.berg@nvh.no	Norwegian School of Veterinary Science
Borge	Thomas	tbo@molegenetics.com	Mole Genetics
Brekke	Helge	helge.brekke@thermofisher.com	Fisher Scientific
Cantas	Hasan	hasan.cantas@nvh.no	Norwegian School of Veterinary Science
Collas	Philippe	philc@medisin.uio.no	University of Oslo
Dahlbom	Josefin	josefin.dahlbom@neuro.uu.se	Uppsala university, Neuroscience
Deng	Wei	wei.deng@sars.uib.no	Sars International Centre for Marine Molecular Biology
Ellingsen	Ståle	sel@nifes.no	NIFES
Evensen	Oystein	oystein.evensen@nvh.no	Norwegian School of Veterinary Science
Fenaroli	Federico	federico.fenaroli@imbv.uio.no	Uio
Flack	Aksel	aksel.flack@roche.com	Roche Diagnostics Norge AS
Greve	Ina Killingrød	ina.greve@nvh.no	NVH
Griffiths	Gareth	garetg@imbv.uio.no	IMBV UiO
Gupta	Puja	emailpuja@gmail.com	University of Bergen
Hagen	Anita	ah@scanbur.eu	Scanbur AS
Havre	Trond	trond.havre@olympus.no	Olympus Norge as
Hegge	Beate	beate.hegge@uit.no	Farmakologi, IFA, Universitetet i Tromsø
Helvik	Jon Vidar	jon.helvik@bio.uib.no	University of Bergen
Hildahl	Jon	jon.hildahl@nvh.no	Norwegian School of Veterinary Science
Hultman	Maria	maria_therese_hultman@hotmail.com	Norwegian Institute for Water Research (NIVA)
Høibakk	Arne	arne.hoeibakk@olympus.no	Olympus Norge As
Joung	J. Keith	jjoung@partners.org	Massachusetts General Hospital and Harvard Medical School
Juthajan	Aphirak	aj@zeiss.no	Carl Zeiss AS
Kraugerud	Marianne	marianne.kraugerud@nvh.no	Norwegian School of Veterinary Science
Kreneisz	Orsolya	orsolya.kreneisz@sars.uib.no	Sars International Centre
König	Melanie	melanie.koenig@nvh.no	NVH
Lindeman	Leif	l.c.lindeman@medisin.uio.no	Institutt for medisinske basalfag, Universitetet i Oslo
Lock	Erikjan	elo@nifes.no	NIFES
Male	Rune	rune.male@mbi.uib.no	University of Bergen, Department of Molecular Biology
Midtlyng	Paul J	paul.midtlyng@nvh.no	Norwegian School of Veterinary Science
Mikkola	Ingvild	ingvild.mikkola@uit.no	Department of Pharmacy, University of Tromsø
Moen	Lars	lars.moen@nvh.no	Norwegian School of Veterinary Science
Müller	Ferenc	f.mueller@bham.ac.uk	University of Birmingham

Neuzeret	Didier	info@viewpoint.fr	ViewPoint
Nordeide	Hildegunn	hildegunn.nordeide@sial.com	Sigma-Aldrich
Panula	Pertti	pertti.panula@helsinki.fi	Institute of Biomedicine and Neuroscience Center
Penglase	Sam	spe@nifes.no	Nifes
Peterson	Trace	fishpathman@gmail.com	Oregon State University
Phillips	Siân Kristin	sian.phillips@mbi.uib.no	Institute for Molecular Biology, UoB
Piston	Dominik	dominik.piston@uis.no	University of Stavanger, Faculty of Mathematics and Natural Science, CORE
Reinshol	Øyvind	oyvind.reinshol@nifes.no	NIFES
Ropstad	Erik	erik.ropstad@nvh.no	Norwegians School of Veterinary Science
Rusten	Marte	martte.rusten@mbi.uib.no	Molekylærbiologisk Institutt, Universitetet i Bergen
Sankala	Marko	marko.sankala@sial.com	Sigma-Aldrich
Straehle	Uwe	uwe.straehle@kit.edu	Karlsruhe Institute of Technology
Strandabø	Rønnaug	ronnaug.strandabo@imbv.uio.no	University of Oslo, Department of Molecular Biosciences
Sulen Tavara	Ana Carolina	Ana.Tavara@nvh.no	Norwegian School of Veterinary Science
Suster	Maximiliano	maximiliano.suster@sars.uib.no	Sars International Centre for Marine Molecular Biology
Sørby	Jan Roger Torp	jan.roger.torp@nvh.no	Norwegian School of Veterinary Science
Sørensen	Christina	christina.sorensen@imbv.uio.no	Universitetet i Oslo
Thorsteinsson	Haraldur	haraldurth@ru.is	Reykjavik University
Tollefsen	Knut Erik	ket@niva.no	Norwegian Institute for Water Research (NIVA)
Ul Haq	Mazhar	mazhar.ulhaq@bvf.slu.se	SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES
Ulanova	Lilia	lilia.ulanova@imbv.uio.no	University of Oslo
Valen	Ragnhild	ragnhild.valen@bio.uib.no	Institute of Biology, University of Bergen
Velde	Håkon	hve@ahdiag.no	AH diagnostics
Weltzien	Finn-Arne	finn-arne.weltzien@nvh.no	Norwegian School of Veterinary Science
Zhao	Xiang	xiang.zhao@helsinki.fi	University of Helsinki
Østli	Even	even@matriks.no	Matriks AS
Aanes	Håvard	havard.aanes@nvh.no	NVH