Nordic Zebrafish Meeting 2024

From husbandry to animal experiments: Creating reliable research

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ABSTRACT BOOK

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Abstracts of keynote and short talks

Harmonizing Research for the 3Rs: Tailoring zebrafish husbandry for their purposed research

Gregory Paull[1]

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Gregory Paull is the manager of the Aquatic Resources Centre, a large interdisciplinary research facility which supports over 100 scientists whose studies span environmental and human health, aquaculture and understanding biological systems. Greg has worked at the University of Exeter, UK for over 20 years where he also obtained his PhD in fish reproductive biology and eco-toxicology, with zebrafish as one of his key study species. Greg has had the fortune to study zebrafish in their natural environment and champions understanding of their natural history to support in-laboratory husbandry, care and welfare. Greg is also a Named Animal Care and Welfare Officer.

Zebrafish are often described as an easy to keep species, however this is far from being the case when keeping this species in the laboratory for different and diverse research purposes and needs. In my talk, I will describe the various challenges faced for the application of zebrafish in a number of key research areas. I will then illustrate how the lack of standardisation of husbandry practices/approaches in the zebrafish research community is contributing to uncertainties in research data and in some cases contributing to poor experimental reproducibility. I will call on the 'zebrafish community' to help develop current husbandry practices, which are often in the most basic form, to better suit their research use and the animal's welfare. Finally, I will discuss some ideas/concepts and identify existing expertise to help achieve practical developments in zebrafish husbandry that better align with their research use.

Packing and transport of adult zebrafish cause both acute and long-term elevated water cortisol levels

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Zebrafish are often transported both short and long distances, for example, when being collected in the wild, or when shipped between laboratories. Despite this, it is still not fully known how transporting zebrafish affects their welfare. To better understand the short- and long-term physiological and welfare effects of transporting zebrafish, we performed a series of experiments transporting zebrafish.

Adult wildtype zebrafish were raised at a zebrafish core facility in Stockholm and transported to our research facility in Gothenburg, a journey that took around 6 hours by car. Animals were packed for the transport in plastic bags (20 fish per bag) according to commonly used standard operations procedures. Upon arrival, the transport water was collected for analysis of water quality and content of the stress hormone cortisol that was measured using a radioimmunoassay. After being acclimatized to the new facility the fish were transferred to tanks with fresh aquarium water, and kept up to nine days during which the water was collected for analysis.

Our experiments showed that water cortisol levels in the transport bags after the transports were substantially higher than cortisol levels normally measured in their aquaria. One day after the transport, the cortisol levels showed large variations between the home tanks, but had dropped to about a tenth of the transport concentrations. After the transport experiment, the water cortisol levels remained elevated until five days, when they were significantly lowered. This indicates that packing and transporting of adult zebrafish induce an elevated secretion of cortisol in the water, which could be a sign of both acute and long-term stress.

More research is needed to better understand the welfare implications of the elevated cortisol, but we foresee that our findings could have implications for the aquaculture and transport of other fish species, for example, farmed or ornamental fish.

Transgenerational epigenetic inheritance of neurobehavioral alterations in zebrafish caused by environmental pollution

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Research has shown that pollutants pose significant risks to human and environmental health, with recent studies linking these risks to epigenetic changes passed through generations. Environmental factors, including nutrients, stress, and chemical exposure, can induce the inheritance of specific phenotypes. For example, in zebrafish, early-life stress reduces anxiety-like behaviors in later offspring, while ancestral exposure to pollutants like DDT has been linked to obesity and behavioral changes in rodents.

Our research demonstrates that ancestral exposure of zebrafish to environmentally relevant concentrations of PFAS altered neuro-phenotypes in unexposed generations. Specifically, F2 zebrafish showed behavioral changes linked to transcriptional and DNA methylation alterations in neurodevelopmental genes. In another study, zebrafish exposed to the insecticide permethrin during early life exhibited transgenerational neurotoxic effects. F0 exposed fish displayed hypoactivity in adulthood, while unexposed F1 and F2 males showed reduced anxiety-like behavior. Transcriptomic and epigenomic analyses revealed persistent dysregulation of glutamatergic synapse activity and stable inheritance of differentially methylated regions from F0 to F2.

Together, these findings highlight the role of epigenetic mechanisms, such as DNA methylation and transcriptional regulation, in transgenerational inheritance. They also provide new insights into how exposure to environmental pollutants, including PFAS and permethrin, can lead to epigenetic changes and neurobehavioral alterations across generations.

fhl2b mediates extraocular muscle protection in zebrafish models of muscular dystrophies and its ectopic expression ameliorates affected body muscles

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In muscular dystrophies, muscle fibers loose integrity and die, causing significant suffering and premature death. Strikingly, the extraocular muscles (EOMs) are spared, functioning well despite the disease progression. Although EOMs have been shown to differ from body musculature, the mechanisms underlying this inherent resistance to muscle dystrophies remain unknown. Here, we demonstrate important differences in gene expression as a response to muscle dystrophies between the EOMs and trunk muscles in zebrafish via transcriptomic profiling. We show that the LIM-protein Fhl2 is increased in response to the knockout of desmin, plectin and obscurin, cytoskeletal proteins whose knockout causes different muscle dystrophies, and contributes to disease protection of the EOMs. Moreover, we show that ectopic expression of fhl2b can partially rescue the muscle phenotype in the zebrafish Duchenne muscular dystrophy model sapje, significantly improving their survival. Therefore, Fhl2 is a protective agent and a candidate target gene for therapy of muscular dystrophies.

Ependymal status and CSF flow in health and disease

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The brain is supplied with interconnected cavities filled with cerebrospinal fluid (CSF). CSF is produced by the ependymal cells of the choroid plexus, and its circulation is maintained through the synchronized beating of cilia covering ependymal cells (EC) which line the ventricle walls. Proper CSF content and circulation is crucial for nutrient delivery, waste removal, and overall brain health. Previous research has shown that several neurodegenerative diseases, including Alzheimer's disease, are associated with changes in EC and CSF flow. Ependymal status may therefore be a good indicator of functional CSF flow. The intimate contact between EC and CSF makes it likely that changes in ependymal cells can be detected in CSF samples.

We have utilized CSF proteome studies to identify cilia-related proteins in CSF of normal and diseased individuals. We have identified proteins that are changed in individuals with cognitive impairments and Alzheimer's disease and have investigated how these correlates with cognitive decline and other AD biomarkers. Using genetic tools to manipulate gene expression and follow protein localization in zebrafish, we are now addressing the presence of differentially expressed proteins in ependymal cells and their role in ciliogenesis and CSF flow. By determining the underlying molecular mechanisms, we hope to contribute to a better understanding of the disease processes not only in AD but also other neurological disease where cilia or CSF flow may be affected.

Zebrafish models of innate immunity

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Steve Renshaw is the Sir Arthur Hall Professor of Medicine at the University of Sheffield and Head of the Division of Clinical Medicine. He studied medicine at Cambridge and then at Oxford Clinical School. He has been a Wellcome Trust Clinical Training Fellow, an MRC Clinician Scientist Fellow, an MRC Senior Clinical Fellow and an MRC Programme Grant holder. His lab focusses on the biology of innate immune cells, particularly the neutrophil, and their relevance to respiratory disease. His major contribution has been the development of the transparent, genetically-tractable larval zebrafish as a model for the study of innate immunity in vivo. He has developed several unique transgenic zebrafish which have allowed several important advances in our understanding of inflammation biology and of host-pathogen interaction. He continues clinical work in Respiratory Medicine with a special interest in Interstitial Lung Disease associated with a range of multisystem diseases.

Neutrophilic inflammation underpins most of the diseases of ageing which are the scourge of health systems around the world, including common and incurable lung diseases. Despite this, the neutrophil is the target of virtually no therapies, in part due to the intractability of human neutrophils. Inspired to address this gap, we generated a transgenic zebrafish model to aid the study of neutrophil function in vivo, which has proved highly useful for a range of applications. I will discuss some of these in my talk, including identification of new classes of pro-resolution therapeutic with unexpected targets and new understanding of the mechanics of phagocytosis and bacterial killing.

The inflammasome adaptor *pycard* is essential for immunity against *Mycobacterium marinum* infection in adult zebrafish

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Inflammasome regulates the host response to intracellular pathogens including mycobacteria. *Mycobacterium marinum* infection in adult zebrafish *(Danio rerio)* resembles the course of tuberculosis in human, and has been used for studying genes essential for host protective immunity in mycobacterial challenge.Here we have investigated the role of the inflammasome adaptor *pycard* in *M. marinum* infection in zebrafish. Using zebrafish knock-out mutant lines for the *pycard* gene with CRISPR/Cas9 mutagenesis, we studied both larval and adult zebrafish response to a mycobacterial infection. While the zebrafish larvae devoid of *pycard* develop normally and have unaltered resistance against *M. marinum*, the loss of *pycard* led to impaired survival and increased bacterial burden in the adult zebrafish. Based on histological analysis, immune cell aggregates, granulomas, were larger in *pycard* deficient fish compared to wild type controls. Transcriptome analysis with RNA sequencing of the zebrafish haematopoietic kidney marrow, suggests a role for *pycard* in neutrophil mediated defense as well as in haematopoiesis and myelopoiesis during infection. Transcriptome analysis of fluorescently labelled kidney neutrophils further supported the importance of *pycard* for neutrophil-mediated immunity against *M. marinum*. Genes associated with neutrophil degranulation and haematopoiesis were differentially expressed in the *pycard* deficient neutrophils when compared to wild type controls. Together, our results indicate that *pycard* is essential for resistance against mycobacteria in adult zebrafish. Based on transcriptional profiling of *pycard* mutants, we postulate that *pycard* mutant phenotype is mediated in part via defects in neutrophil function including neutrophil degranulation.

How to adapt imaging to your needs

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Host-parasite interactions are inherently complex due to the interplay of biological signals from two distinct organisms. From a molecular standpoint, it is often challenging to determine whether observed changes in host signaling pathways are a result of the host's defense mechanisms or are driven by parasitic manipulation of these pathways. Disentangling this dynamic is particularly difficult because both organisms contribute to the regulation of cellular and molecular processes. Techniques such as metabolomics and proteomics offer valuable insights, but accurate interpretation requires precise knowledge of the origin of each molecular signal—whether from the host or the parasite.

Histological methods and immunohistochemistry can provide a detailed visualization of these interactions, but only ex vivo. In contrast, the zebrafish model offers an excellent platform for in vivo studies, particularly due to its transparency and the availability of transgenic lines with fluorescence markers. These tools enable real-time imaging of host-parasite interactions but requires innovative and creative approaches.

In this work, I will present our imaging strategies for two distinct host-parasite systems in zebrafish. We utilized the protozoan ciliate Ichthyophthirius multifiliis and the trematode Diplostomum pseudospathaceum to explore and visualize their interactions within the host environment. These findings highlight the utility of zebrafish as a model organism and the potential of advanced imaging techniques to reveal the complexities of host-parasite relationships.

Solitary chemosensory cells in zebrafish skin: evidence for a role in host-microbiota interactions

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The skin of many species of fish is peppered with solitary chemosensory cells (SCCs) of unknown function. These cells are characterised by an actin-rich apical protrusion that is in contact with the external environment, while the soma is surrounded by afferent neurites. In some species, SCCs have been observed to respond to an undefined component of fish mucus and human saliva, based on recording of the facial nerve. Here, we use the zebrafish to test the hypothesis that SCCs mediate an interaction with bacteria, which are known reside in these substances. A feature of SCCs, which are found in larval, juvenile and adult stages, is the high content of serotonin. Analysis of single cell transcriptome data from larval skin identified one cluster of cells that expresses genes involved in the synthesis of serotonin. This cluster is characterised by the presence of several neuropeptides including the gastrin-related peptide (*grp*). In situ hybridization indicates that all solitary chemosensory cells express *grp*. The cluster contains the *htr3al* receptor and in situ hybridization confirms that this gene is expressed in SCCs. Calcium imaging demonstrates that SCCs respond to a transient exposure of external serotonin with a sustained rise. Depletion of these cells, using nitroreductase, alters the microbial population in the larval skin, as judged by ARISA. Given that serotonin is a bacterial signalling molecule, we propose that SCCs mediate bi-directional communication between the host and bacteria, influencing host behaviour as well as microbiota composition. SCCs are thus a previously unrecognised component of neuro-immune interactions in the fish.

Designing a Health Monitoring program for zebrafish

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This presentation is about providing insights in developing a health monitoring program for zebrafish. The program must aim to detect both infectious and non-infectious diseases, including pathogenic and non-pathogenic conditions and quarantine procedures to prevent the spread of disease within the colony and to new colonies. We will examine how different sample types are impacting diagnosis and how prevalence and institutional prevalence are important in determining sample size. The goal of this presentation is to contribute to the development of effective measures for the prevention and control of diseases in zebrafish.

Thermal choice and fever in fish

Simon Mackenzie[1]

1. Institute of Aquaculture, University of Stirling, Stirling, United Kingdom

Professor Simon MacKenzie is the Head of the Institute of Aquaculture at the University of Stirling, Scotland. He runs an active and vibrant multi-disciplinary research team engaged in different aspects of immunity, physiology, ecology and disease in fish with an underpinning focus upon molecular regulation. He has spent the past 20 years mainly working upon experimental models relevant to fish health with a particular emphasis upon the evolution of the immune system and health and welfare in aquaculture. Recent work focuses upon gill microbiomes and fish husbandry management, the molecular regulation of smoltification and insect meals as immunomodulators in fish. Currently, Professor MacKenzie is the co-Editor in Chief of Frontiers in Aquaculture a new journal in the aquaculture sphere.
In my talk I will explore the impact of thermal choice upon the behavior of fish highlighting how the immune response is regulated through the fever response. We will discuss how temperature choice has wide-ranging effects upon regulatory systems in fish and how this impacts their welfare.

Zebrafish as a cancer model

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Zebrafish has become an important model organism for cancer research. Their rapid, extrauterine development greatly facilitate genetic manipulation and/or tumor implantation in zebrafish embryos and larvae, and due to the optical transparency of all tissues at these early stages, allow detailed visualization of tumor initiation, development and progression within days. Recently, our lab and others have demonstrated the pathophysiological relevance of studying human tumor xenografts in zebrafish larvae by showing that primary patient tumor tissues engraft in the larvae and respond similarly to anti-cancer therapies as the patients themselves. These finding have led to zebrafish tumor xenograft models taking center stage as a functional precision medicine instrument of high clinical relevance. There are, however, many open questions regarding the use of zebrafish cancer models for both basic tumor biology research and precision medicine including to what extent the tumor microenvironment in the zebrafish models is relevant for understanding for example complex tumor-immune regulations, and to what extent host-derived ligands provide a relevant signaling environment through tumor cell-expressed receptors to study efficacy of for example therapies targeting growth factor receptors.

We have recently started elucidating the experimental conditions affecting tumor-immune interactions to understand how these can be accurately recapitulated within zebrafish xenograft models. Using our new protocols, we show that responses to checkpoint inhibitors as well as autologous T-cell therapies can be identified in a patient-specific manner in only three days. Furthermore, we have designed a personalized drug development platform with the potential to develop a unique, personalized biological therapy for cancer patients, ready for clinical testing, in only 6 months. These and other recent advances in zebrafish tumor xenograft models and their applications for precision medicine will be presented and the existing knowledge gaps and need for further research will be discussed in the talk.

Investigating the role of *MYCN* in neural crest cells

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Neuroblastoma (NB) is an embryonic tumor that originates from cells within the neural crest. Primary tumors are found in the adrenal gland or along the paraspinal ganglia. The chromaffin cells of the adrenal gland were recently found to partly originate from a subset of neural crest cells called Schwann cell precursors (SCP). Amplification of the gene *MYCN* is one of the most common genetic aberrations and a major risk factor for poor prognosis in patients with NB. MYCN expression driven by the Th or dβh promoters results in the development of NB in both mouse and zebrafish models. Both models express MYCN in later stages of the sympathoadrenal development. Our recent scRNA seq data on human NB samples suggests that NB is initiated at earlier stages in the neural crest.

Hence, we investigated early genetic events in the SOX10+ SCPs that could collaborate to development of NB. For this purpose, we have generated transgenic zebrafish with inducible expression of *MYCN* in SOX10+ cells. We injected CMV:LSL-EGFP-P2A-MYCN constructs into *Tg(sox10:creERT2)* zebrafish at the one-cell stage followed by Tamoxifen induction at different timepoints. The fish were screened for tumor development through live fluorescence imaging and H&E/IHC/IF. So far, 8 fish have been sectioned and stained (3 control, 3 induced at 48h and 2 induced at 24h). In the ones induced at 24h we see expression of MYCN in the head kidney, and abnormal growth in the interrenal gland with expression of several NB markers.

Zebrafish are useful models to study early events in NB and with this project we hope to learn more about the mechanisms behind tumor development.

Development of an orthotopic medulloblastoma zebrafish model for rapid drug testing

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Background
Medulloblastoma (MB) is one of the most common malignant brain tumors in children. Current preclinical *in vivo* model systems for MB have increased our understanding of molecular mechanisms regulating MB development. However, they may not be suitable for large-scale studies. The aim of this study was to investigate if a zebrafish-based xenograft model can recapitulate MB growth and enable rapid drug testing.

Methods
Nine different MB cell lines or patient-derived cells were transplanted into blastula-stage zebrafish embryos. Tumor development and migration were then monitored using live imaging. RNA sequencing was performed to investigate transcriptome changes after conditioning cells in neural stem cell-like medium. Furthermore, drug treatments were tested in a 96-well format.

Results
We demonstrate here that transplantation of MB cells into the blastula stage of zebrafish embryos leads to orthotopic tumor growth that can be observed within 24 hours after transplantation. Importantly, the homing of transplanted cells to the hindbrain region and the aggressiveness of tumor growth are enhanced by pre-culturing cells in a neural stem cell-like medium. The change in culture conditions rewires the transcriptome towards a more migratory and neuronal phenotype, including the expression of guidance molecules SEMA3A and EFNB1, both of which correlate with lower overall survival in MB patients. Furthermore, we highlight that the orthotopic zebrafish MB model has the potential to be used for rapid drug testing.

Conclusion
Blastula-stage zebrafish MB xenografts present an alternative to current MB mouse xenograft models, enabling quick evaluation of tumor cell growth, neurotropism, and drug efficacy.

The sex inclusive research framework

Natasha Karp[1]

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Natasha is a Director of Hit Discovery and Biostatistics within AstraZeneca leading a team of statisticians and bioinformaticians supporting preclinical research. In addition, Natasha is an active researcher publishing papers with a focus on the challenges within preclinical research with a particular interest in improving replicability, reproducibility, and generalizability of the studies. In recent years, the research has focused on meta-research exploring how to enable and nudge scientists into better research practice.

In my seminar, I will present a new, interactive sex inclusive research framework (SIRF) which supports the evaluation of in vivo and ex vivo research proposals from a sex inclusive research perspective. The framework delivers a traffic light classification, indicating whether a proposal is appropriate, risky, or insufficient with regards to sex inclusion. This tool is designed for use by researchers, (animal) ethical review boards, and funders to generate a rigorous and reproducible assessment of sex inclusion at the proposal level, thus helping address the embedded sex bias in preclinical research.

Zebrafish as a Long QT Syndrome model for drug screening

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The zebrafish (*Danio rerio*) model is proving to be a relevant model in the search of drugs with anti-arrhythmic potential. Zebrafish are ideal for drug screening due to their small size, rapid development, and high fecundity. In addition, the zebrafish is an emerging model for cardiac research because it shares interesting electrophysiological characteristics, particularly the shape of the ventricular action potential is very similar to that of humans. Our recent studies have shown that the zebrafish is a good animal model for testing small molecules that activate the cardiac KCNQ1/KCNE1 (I_{Ks}) channel.

The aim of this project is to generate zebrafish embryos with a variety of channel mutations that cause long QT syndrome (LQTS) and cardiac arrhythmia and then test small-molecule I_{Ks} activators on these LQTS zebrafish embryos. To this end, we are developing an approach consisting of injecting wild-type (wt) or mutant hKCNQ1/hKCNE1 mRNA into 1-cell stage embryo. We hypothesize that mRNA encoding hKCNQ1/hKCNE1 mutated will prolong the action potential duration (APD), leading to LQTS phenotypes, and that our small-molecule I_{Ks} activators will reduce this prolonged APD, rescuing normal cardiac function.

By understanding how various arrhythmia-causing mutations lead to dysfunctional ion channels and how our small-molecule modulators restore their function, we aim to develop more personalized treatments for patients with cardiac arrhythmias. The zebrafish model can allow us better characterization of specific LQTS variants that are challenging to assess using standard electrophysiology techniques. Additionally, this *in vivo* high-through screening platform approach holds promise for advancing more targeted and individualized therapeutic strategies, which are crucial for better managing cardiac arrhythmia diseases.

Zebrafish models for cardiovascular biology

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Cardiovascular diseases are globally one of the leading causes of death. Better understanding on the biology of the cardiovascular system would enable development of better therapeutics. This, however, requires the use of efficient research models. Zebrafish models have gained popularity in the study of cardiovascular biology and drugs. We have utilized various zebrafish models to understand cardiovascular development, biology and disease. These include modeling of heart failure, myocardial ischemia, cardiotoxicity of chemicals and analysis of development of cardiovascular system during zebrafish embryogenesis.

A deep learning method to quantify intersegmental vessels in zebrafish embryos

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Zebrafish embryos expressing fluorescent vessels are widely used for angiogenesis studies due to their transparency and genetic relevance to humans. However, manual segmentation and quantification of these vessels remain a bottleneck, especially in large-scale studies. While deep learning models show promise in automating general zebrafish vasculature segmentation, there is a notable gap in models for segmenting the intersegmental vessels that are crucial for angiogenesis studies. Here, we developed an automated method using deep learning and morphological operations to segment and quantify intersegmental vessels in zebrafish embryos. We compared two models: U-Net, known for medical image segmentation with smaller datasets, and MedSAM, known for handling more complex image structures. Ground truth masks were manually annotated from one of three batches of images and used to train, validate, and test both models. U-Net outperformed MedSAM in segmentation accuracy, achieving a higher Dice Similarity Coefficient and intersection-over-union scores, making it the preferred model for downstream analysis. Morphological operations were then applied to the U-Net segmentations of the other two batches to extract vessel metrics, such as height, number, width, and area. Analysis of intersegmental vessels at 8 hpf and 24 hpf revealed significant differences, demonstrating the model's ability to quantify developmental changes. This automated approach can reduce the time and manual effort required for zebrafish vascular analysis, which will increase consistency and scalability for larger datasets. We are now developing a user-friendly tool to enable non-experts in image analysis to apply automated quantification in other zebrafish studies of angiogenesis.

Phagocytosis of photoreceptor outer segments by retinal pigment epithelium imaged in living zebrafish

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Light absorbing rod and cone photoreceptor cells at the back of the eye in the retina depend on retinal pigment epithelium (RPE) to maintain their viability. RPE helps to renew photoreceptors' light absorbing outer segments (OS) by phagocytosing the aged parts that contain harmful, photo-oxidative waste products. Failure in this interaction is often the underlying cause for several retinal diseases. RPE phagocytosis is a complex process but detailed understanding on the dynamics of OS particle intake and degradation within RPE as well as differences between photoreceptor types is presently inadequate hindering the development of effective treatments. Previously, RPE phagocytosis has been studied with isolated *ex vivo* tissues or cultured cells without physiological context within an entire animal. For the first time, we present RPE phagocytosis event imaged in a living animal.

Zebrafish is one of the most accessible models to study retinal physiology *in vivo*. They have four cone photoreceptor types in addition to one type of rods providing an excellent model to study the possible differences in phagocytosis between photoreceptor types. By utilizing already existing transgenic lines and microinjecting photoreceptor and RPE cell membrane targeted plasmid constructs, we have created low-pigmented fish with fluorescently tagged RPE cells and photoreceptor OS. We present our approach and latest results of time-lapse confocal imaging on an anesthetized zebrafish to show how an OS particle moves from photoreceptor outer segment deeper into an RPE cell until vanishing upon degradation. Our results provide a new approach to study RPE-photoreceptor interactions within a living animal, with increasing possibilities with other tissues as we all as eye disease modelling.

Abstracts of posters

Poster 1:

Investigating the impact of chemically induced neurodevelopmental disorders on gut health

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Neurodevelopmental disorders are strongly associated with disruptions of intestinal function, however the implication of the gut-brain axis remains unclear. We investigated the role of valproic acid (VPA), an anticonvulsant which is widely used as a chemical inducer of neurodevelopmental disorders in animal models, to assess the impact of VPA on gut health and immune cells of the brain. We compared treated germ-free (GF) fish with conventionalized animals at 5 and 7 dpf by which timepoints the digestive system is under development and fully functional, respectively. We also infected the fish with *Vibrio cholera* to investigate the impact of an opportunistic pathogen that rapidly colonizes the gut. Our preliminary data shows that the number of goblet cells was significantly decreased in VPA-treated fish at 5 dpf compared with untreated groups, however this effect was not observed in infected fish treated with VPA. We looked at the effect of VPA on gene expression in the gut in GF animals at 5 and 7 dpf and found that the mucin 5AC like gene (LOC101882681, padj < 0.001) was significantly decreased in VPA-treated GF fish compared with untreated GF fish. At 7 dpf, phox2bb, which is involved in the development of the enteric nervous system and sympathetic ganglions was significantly downregulated in VPA-treated GF fish compared to untreated GF fish (padj < 0.01). Our results suggest that VPA affects gut health at the mucosal level, including the nervous system, and that *Vibrio cholera* bacteria abrogates goblet cell hypoplasia as a result of VPA treatment. Future studies will aim at investigating how VPA affects gut motility and how it is affected by inflammation and the gut microbiota.

Poster 2:

Using zebrafish as a model to study the worm migration: 2D/3D visualization and immune response analysis

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The metacercariae of digenean trematode, commonly known as “eye flukes”, are prevalent in freshwater fish populations, typically residing within the lens of their hosts. Previous studies have highlighted the potential immunosuppressive effects caused by eye fluke infections. However, the specific mechanisms and dynamics of the parasite's migration during its diplostomule stage remain largely unexplored. To investigate this process, the zebrafish (*Danio rerio*) has been employed as an ideal model to study the migration route of the parasite and the corresponding immune responses.

In this study, we use transparent TraNac and Double Transgenic (DT) zebrafish line, along with the eye fluke species *Diplostomum pseudospathaceum*, to establish a parasite-host interaction model. To visualize the migration route and create 3D imaging, we employed two different fluorescent dye: BODIPY 558/568 C12 for staining the parasite, and Carboxyfluorescein Succinimidyl Ester (CFSE) for labeling the zebrafish. Fluorescence stereomicroscope and laser scanning confocal microscope were used to capture detailed images. Additionally, wild-type zebrafish were utilized to access immune responses at different infection time.

This research aim to elucidate the migration behavior of *D. pseudospathaceum* within its fish host and provides a potential model for studying the complex mechanisms of parasites.

Poster 3:

The effect of AhR agonists on zebrafish regeneration and immune function

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The skin epithelium serves as the first line of physical and immunological defense against environmental stressors, including environmental pollutants. In zebrafish, the mechanosensory organs on the skin surface, known as neuromasts, undergo continuous cellular proliferation and regeneration, processes that can be easily disrupted by environmental pollutants. Neuromasts are essential for key behaviors such as orientation, predator avoidance, foraging, and mating; thus, any disruption in their function can have detrimental effects on the entire organism.

The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor involved in cell proliferation, stemness maintenance, and cell cycle progression. Our findings showed that specific chemicals could activate the AhR in the neuromast cells. Furthermore, a variety of environmental pollutants are recognized as AhR activators and are particularly toxic to zebrafish. In this study, we investigate whether exposure to various AhR activators disrupts the established regenerative processes of neuromasts following induced cell death.

We selected 1,2,3-trichloro-5-(3,4-dichlorophenyl)benzene (PCB126) and 3-methylcholanthrene (3-MC) as toxic environmental pollutants, alongside Indolo[3,2-b]carbazole (ICZ) as a non-toxic, non-persistent dietary ligand. Our results indicated that following copper sulfate-induced neuromast cell death, AhR activators exert distinct effects on the macrophage recruitment. Specifically, 3-MC inhibited macrophage recruitment, while PCB126 caused a retention of the macrophages at the damage site. Furthermore, both compounds affected cellular proliferation within the neuromast, with 3-MC causing a pronounced inhibitory effect.

This ongoing study aims to elucidate how exposure to AhR ligands impacts regenerative and immune processes in zebrafish.

Poster 4:

Zebrafish-Based Tumor Xenograft Model for Rapid, Translational Insights into Patient-Specific Treatment Strategies

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As our understanding of cancer therapies advances, it is becoming clearer that there are two arising areas of interest. First, a need for rapid, efficient and biologically relevant screening of potential oncological compounds, and second, the need for personalized medicine strategies to improve patient outcome and quality of life. Current methods exhibit drawbacks that can make them unsuitable for this purpose: Cell models alone may not be biologically relevant to assessing both anti-cancer and anti-metastasis properties of compounds and often requires additional microfluidic chambers or synthetic 3D-matrices, while mouse models are prohibitively expensive for mass screening of patient samples.

Here we describe a zebrafish-based tumor xenograft model that provides the much-needed balance of providing rapid and patient-translational data, at the fraction of resource cost of mice models. This method is suitable for a range of different strategies, including small molecules and ADC, absorption uptake and direct IV injection, as well as immune-oncological therapies. Patient-derived tumor samples are enzymatically processed into a dissociated cell suspension and implanted into 48 hpf zebrafish larvae, while maintaining cell viability, tumor heterogeneity and cellular characteristics. The same method can also be utilized with PDX-models and cell lines, making the zebrafish-based tumor xenograft model a powerful tool for oncology research.

Using this model, we can, for example, perform immune-oncology studies using patient-derived tumor cells additionally with patient-derived lymphocytes to test the efficacy of anti-cancer antibodies on tumor reduction and metastasis, as well as immune cell tracking within the tumor microenvironment. In just three days post-implantation, our verified and validated system will give accurate and translatable data based upon tumor size and metastatic dissemination. Our studies, therefore, present a unique opportunity to yield in vivo patient-specific efficacy data for use in developing novel cancer therapies and for providing new insight in developing personalized medicine strategies.

Poster 5:

Anticonvulsant activity and toxicity studies of xanthones and benzoates from bark of *Securidaca longipedunculata* in zebrafish *Danio rerio*

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Securidaca longipedunculata Fresen (Polygalaceae) is a medicinal plant with a long history in African traditional medicine. This multi-purpose plant, called the mother of all medicines, is used to treat various diseases such as epilepsy by the traditional health practitioners in different African countries [1, 2]. Epilepsy is a neurological brain disorder that involves spontaneous recurring seizures, affecting over 70 million people worldwide. Today's pharmacotherapy does not manage to treat one-third of epilepsy patients, despite the availability of numerous anti-seizure drugs in the western medical market. Furthermore, modern antiepileptic drugs are often characterized with many side effects [3]. Due to the need for optimization of the current epilepsy treatment with good safety profile, this study has investigated the *in vivo* toxicity effects and anti-seizure activity of extracts and compounds from the bark in a zebrafish chemical seizure model. Powdered bark of *S. longipedunculata* was extracted on an Accelerated Solvent Extraction system (ASE) with dichloromethane (DCM). Isolated compounds were identified by one- and two-dimensional NMR spectroscopy. Xanthones and benzyl benzoates were isolated as the major ingredients. The toxicity study aimed to find the maximum tolerated concentration (MTC) of each compound and extract, as well as to evaluate the phenotypic and neurobehavioural effects against the wildtype zebrafish (*Danio rerio*). The potential anticonvulsant activity was assessed by tracking the locomotor behavior for convulsions in acute PTZ (pentylenetetrazol) treated larval zebrafish. The ethanol extract, benzyl benzoate and benzyl-2-hydroxy-6-methoxy-benzoate showed a dual effect, enhancing the locomotor activity of PTZ-treated zebrafish larvae while reducing the locomotor activity of nontreated ones (paradoxical excitation). Further, the study led to the isolation of novel lead compounds for the development of novel antiepileptic drugs (1,7-dihydroxy-4-methoxyxanthone and 2-hydroxy-1,7-dimethoxyxanthone) and $GABA_A$ receptor ligands/modulators (benzyl benzoate and benzyl-2-hydroxy-6-methoxy-benzoate). Future studies should follow up our interesting findings.

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Poster 6:

Defining the molecular mechanisms downstream of Vegfc-Vegfr3 signaling regulating endothelial cell sprouting

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Endothelial cell (EC) sprouting and migration are integral processes in the formation of functional networks, for both blood and lymphatic vasculature. In zebrafish trunk the secondary sprouts from posterior cardinal vein (PCV) give rise to two cell populations, lymphatics and veins, both driving by the Vascular endothelial growth factor c (Vegfc) – Vascular endothelial growth factor receptor 3 (Vegfr3) signalling. This raises the question from when on the different cellular and molecular phenotypes can be observed. To date it has been shown that lymphatic progenitor cells start expressing *prox1a* prior to departure from PCV, and this expression can be switched off to acquire venous phenotype. However, the underlaying cellular heterogeneity of secondary sprouts remain to be determined.

Here we have used live imaging of zebrafish trunk to investigate the dynamics of secondary sprouts. We observed a degree of heterogeny in the lymphatic sprouts that utilize two modes of migration. Using single-cell RNA sequencing we uncovered the molecular signature of the secondary sprout. As they enriched with mechanosensing Ca^{2+} channel, we used live imaging to observe Ca^{2+} dynamics and identified active Ca^{2+} firing in secondary sprouts that diminished towards the end of migration. We showed that blocking ERK activation before sprouting reduces the Ca^{2+} activity, suggesting Ca^{2+} dynamics is potentially downstream of Vegfc-Vegr3 signalling. Taken together, our characterising of migration behaviour and molecular mechanism regulating the process will contribute to our knowledge of how pro-lymphangiogenic inputs induce differential EC behaviour.

Poster 7:

Unraveling host regulation of gut microbiota through the epigenome–microbiome axis

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The influence of the gut microbiome on host physiology, including metabolism, immune function and behavior, is being understood in increasing detail, but less is known about how the host is actively involved in manipulating its microbiome. Recent examples of dynamic interactions between a host's epigenotype and the composition or activity of its associated gut microbiota suggest the opportunity of the host to shape its microbiome through epigenetic feedback mechanisms in response to environmental or microbial cues. We suggest that a bidirectional "epigenome–microbiome axis" is emerging, which embeds environmentally induced variation, and which may influence the adaptive evolution of host-microbe interactions. Our preliminary results indicate associations between changes in DNA methylation and gut microbiome composition in zebrafish. Furthermore, we present an experimental framework for how host methylomes can be precisely edited using a CRISPR/Cas system fused to various epigenetic effectors to potentially shape microbiome-induced phenotypes in a zebrafish model. Such host epigenetic control could facilitate maintenance of intestinal homeostasis, selection for microbial traits influencing host growth, reproduction or disease resistance (fitness), and thus ultimately the evolution of symbiosis

Poster 8:

Investigating the role of smoothened mutations in zebrafish somite and cartilage development

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Zebrafish somite formation is driven by hedgehog signaling activity, and aberrations in somite formation are a classic readout of hedgehog activity during development. Hedgehog signaling activity also drives cartilage formation and ossification in zebrafish jaw and cranial bones early in development- a process similar to human endochondral ossification. Furthermore, the molecular processes that drive chondrocyte hypertrophy during endochondral ossification are pathologically reactivated in the diseased cartilage of osteoarthritic joints. These molecular similarities allow us to utilize the somite morphology and developing cartilage of larval zebrafish to investigate the role of mutations of the Smoothened (SMO) protein, a key component of the hedgehog signaling pathway, on downstream hedgehog signaling. Smoothened is a GPCR-like protein containing a cholesterol binding site in the extracellular cysteine rich domain. A mutation in this domain (referred to as SMO^{R173C}) in humans is highly correlated with increased risk of the development of hip osteoarthritis, but the precise mechanism is still unknown. Our lab generated human *SMO* mRNA constructs that contain the wildtype amino acid sequence, SMO^{R173C} sequence, or mutations of the extracellular domain that are known to abolish cholesterol binding and downstream hedgehog activity and microinjected these mRNAs into freshly fertilized zebrafish eggs laid by $smo^{+/-}$ pairs. Genetic $smo^{-/-}$ zebrafish have severe morphological defects due to the lack of hedgehog signaling. Therefore, we analyzed the morphological rescue of by assessing the overall body morphology and somite shape, as well as the col2a1 protein expression in early chondrocytes. Interestingly, human SMO^{WT} and SMO^{R173C} mRNA can rescue near normal and normal morphological phenotypes (respectively) and normal somite formation, but do not rescue normal cartilage development. These results suggest that there may be different structural or concentration requirements for SMO protein in zebrafish chondrocytes than compared to other tissue types, such as the mesodermal tissues of the somites.

Poster 9:

Effects of halogenated pollutants on zebrafish embryo transcriptome and immune function

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The Baltic Sea is one of the most polluted bodies of water globally, with its wildlife suffering from long-term health issues such as decreased fertility and increased mortality. Although the negative health effects in wild fish are well documented, the mechanisms and chemical drivers behind these effects remain poorly understood. To address this gap, we used the zebrafish embryo model to investigate molecular and functional changes following exposure to a technical mixture of nine organohalogen compounds found in the serum of wild-caught Baltic Sea salmon. To mimic the salmon's exposure, we designed an internal dose regimen that replicated the relative proportions of these compounds in the zebrafish. Transcriptomic analysis revealed dose-dependent disruptions in immune function and metabolism, overlapping with the adverse health effects observed in wild Baltic Sea fish. We also identified likely chemical effect drivers by comparing the gene expression responses induced by the mixture to those elicited by its individual components. In agreement with our transcriptomic findings, we observed a reduction in total macrophages and a dose-dependent suppression of tissue damage responses in zebrafish. This study provides important insights into how chemical pollutants affect the health of Baltic Sea wildlife.

Poster 10:

The Zebrafish Core Facility at Karolinska Institutet

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The zebrafish core facility at Karolinska Institutet is one of the largest service-providing zebrafish facilities in the Nordic Countries.

Our services range from pure husbandry of zebrafish lines to the management of complex research projects including planning of all experiments, executing of the study and data collection.

We provide quality controlled and standardized experimental pipelines for diverse research areas such as translational medicine, cancer modelling, toxicology, immunology and infection biology, generation of transgenic and/or knock-out animals, behavior studies and much more.

The zebrafish core facility is organized in a way that animal experiments in embryonic zebrafish can be performed, and data be delivered within a mere seven days after ordering. The facility holds all necessary ethical permits and provides the most commonly used wildtype and transgenic lines for immediate use. If needed, the core facility assists in writing ethical permits.

The zebrafish core facility can be contacted by emailing to zebrafish-office@km.ki.se and our webpage can be found here:

Poster 11:

The cancer drug discovery pipeline provided by the zebrafish core facility at Karolinska Institutet

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The zebrafish core facility at Karolinska Institutet is one of the largest service-providing zebrafish facilities in the Nordic Countries.

Amongst others, the facility specializes in cancer research and cancer drug discovery using the zebrafish model.

We provide standardized and quality-controlled pipelines for allotopic or orthotopic transplantation of cancer cell lines or primary material into zebrafish embryos. We are able to implant cancer cells into the cardiovascular system, into the central nervous system or into the perivitelline space of zebrafish embryos.

Hundreds of embryos can be implanted per hour, which allows for drug profiling and high-throughput screening. For the latter, we provide automated high-throughput imaging using a microscope specifically designed for zebrafish in vivo imaging. As the imaging is performed in 96-well plates, every single transplanted embryo is followed individually during the course of the experiment which provides superior statistical power.

For more information on our cancer-discovery pipelines, please visit the poster or come and talk to us. You can even reach us by emailing zebrafish-office@km.ki.se or by visiting our website:

Poster 12:

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High-throughput pipeline for the zebrafish embryonic cancer xenotransplantation

Cancer modeling in zebrafish system is increasingly used by reserchers and biotech companies to accelerate drug discovery and personalize medication of the cancer patients. The zebrafish embryo allows for xenotransplantations with phenotypic evaluation of tumor growth, micrometastasis, blood vessels infiltration and interactions between cancer cells and host macrophages. However, this developing area of research lacks golden standard procedures for image acquisition and quantification, which makes results difficult to evaluate.
Using an automated image acquisition system, we are establishing a multipurpose imaging and machine learning-based image analysis pipeline. The pipeline is currently evaluated through the following projects:
1) Resistance mechanisms of breast cancer cells to the cyclin-dependent kinase 4/6 inhibitor, Palbociclib, which is routinely used to treat patients with metastatic breast cancer.
2) The role of NDSTs, the key heparan sulfate modifying enzyme in glioblastoma (GBM) progression. The usual *in vivo* mouse models for GBM has methodological problems and the tumor take for the human cells in the immune-deficient mice lacks accuracy.
In these projects, the zebrafish model is used to either complement the in vitro obtained data or partially replace rodent models. Our aim is to establish a high-throughput, unbiased, quantitative pipeline which will enable usage of the zebrafish xenotransplantation model for multiple applications and strengthen reliability of the results.