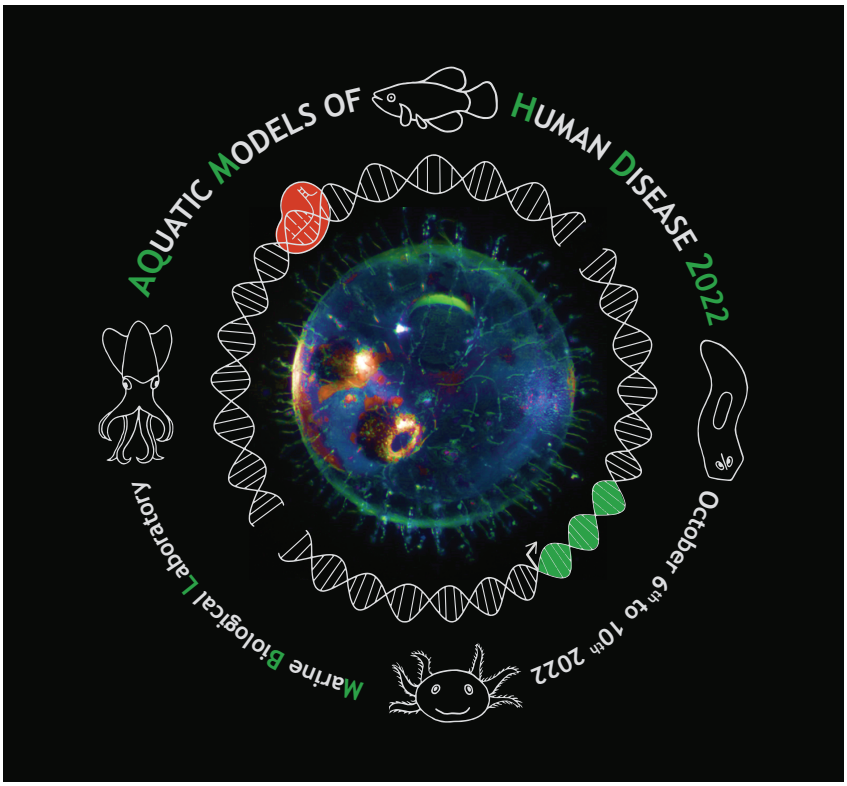
# 10th AQUATIC MODELS OF HUMAN DISEASE CONFERENCE 2022

**October 6 – 10, 2022**

**Marine Biological Laboratory**




## Organizing Committee:

Ingo Braasch (Michigan State University)
braasch@msu.edu

Matthew Harris (Boston Children's Hospital & Harvard Medical School)
harris@genetics.med.harvard.edu

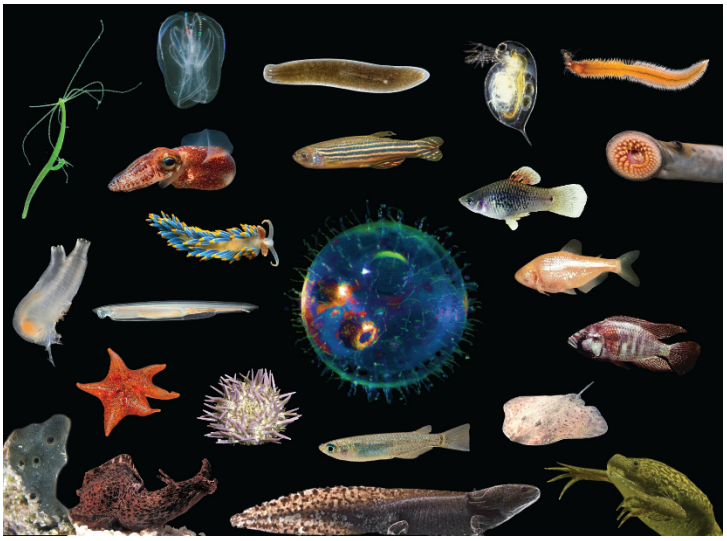
Frauke Seemann (Texas A&M University - Corpus Christi)
Frauke.Seemann@tamucc.edu

Patricia Schneider (Louisiana State University)
pschneider@lsu.edu

## Local Organizer:

Marko Horb (Xenopus Stock Center, MBL)
mhorb@mbl.edu

## A Warm Welcome to AQMHD 2022 and the MBL in Woods Hole



We are very proud to announce the return of the AQMHD meeting after the pandemic enforced interruption at the MBL, the oldest marine laboratory in the United States. Your willingness to travel despite continuing pandemic-related challenges to support our aquatic models community and to re-connect with colleagues and friends is very much appreciated. Thank you!

This year's meeting is focusing on emerging systems in which to study developmental mechanisms and to develop tools to approach biomedical and clinical research. Many aquatic animals have unique advantages and attributes that make them superior choices compared to mammalian models to investigate complex scientific questions. This year's conference features innovative studies in more than 30 aquatic research organisms and provides a platform for three workshops showcasing *Genomic Tools for Aquatic Models, Aquatics Nutrition and Reference Diet Development*, and *Cryopreservation and Open Hardware.*

Platform presentations and lightning talks were designed to highlight the research of new investigators and students, and to support them with ideas, background, and mentoring to enable their success, furthered by the *NIH Peer Review Info Session* and our keynote speakers. We also welcome the attendees of the MBL SCARE Course to our joint sessions on Regeneration.

Proceedings from this meeting will be published in a special issue of the *Journal of Experimental Zoology B: Molecular Developmental Evolution*.

We would like to thank our multiple sponsors, which actively engange with and support our Aquatic Models of Human Disease community through a info session and vendor presence.

We wish you enriching discussions, novel collaborations, and a succesful conference!

*Sincerely,*

Patricia Schneider, Ingo Braasch, Frauke Seemann, and Matthew Harris

# Sponsors

**Award: 1R13OD030023-01**

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## Platinum Sponsors

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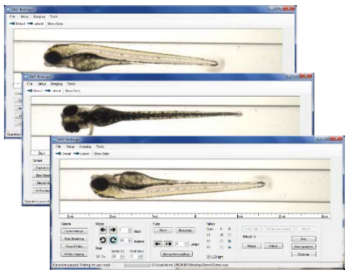
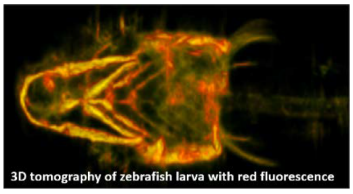
## VAST BioImager™ Platform

Automates positioning of 2-7 dpf larvae reducing the time-consuming and tedious steps of manual manipulation of each larva during large imaging screens.

- Loads each larva into a capillary, positions and rotates them reproducibly to the desired orientation.
- Organ-level imaging with on-board 10µm resolution bright-field camera.
- Mounts on most upright high resolution fluorescent microscopes for cellular-level imaging.



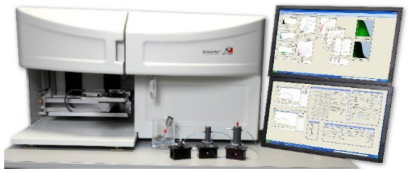
## VASTomography™ Software Enables 3D Zebrafish Research



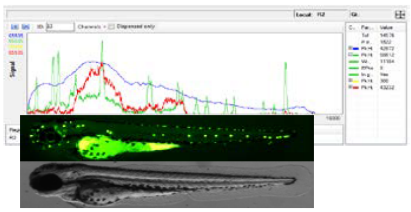

Software generates optical projection tomographic reconstruction (OPT) which builds a 3D density model of the fish larvae based on light transmission through the sample.

- Generate 3D optical projection tomography (OPT) in minutes
- Data sets of volume, shape and position for selected regions of interest
- 3D visualization with VolView/ParaView
- Data processing with MATLAB/Python

## BioSorter® Large Object Flow Cytometer with Profiler™



**BioSorter** -- Gentle analysis and sorting of objects which are too large or too fragile for traditional flow cytometers makes this an ideal tool for dispensing eggs & larvae into multi-well plates.



**Profiler** -- Digitizes optical density and fluorescence signals along the axial length of the object as it passes through the flow cell.

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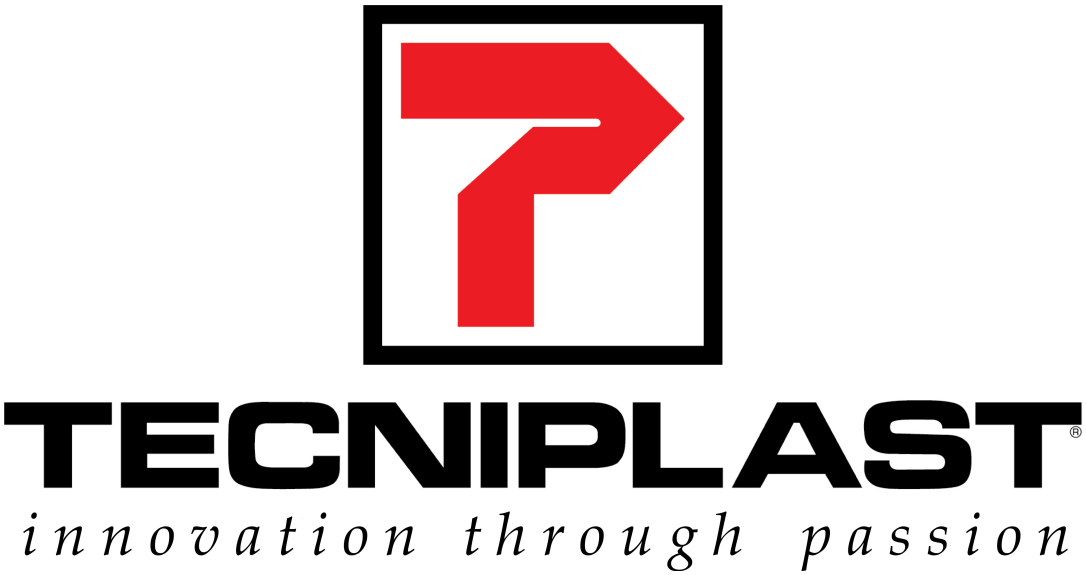


## HCR RNA-FISH: simple, scalable, superior in situ hybridization

In situ hybridization is an invaluable tool for life sciences research, allowing researchers to visualize anatomical distribution and localization of DNA and RNA targets. However, existing in situ hybridization methods are unable to perform robust analyses of gene expression, especially in thick, autofluorescent tissue samples. At Molecular Instruments (MI), our goal is to make in situ hybridization routine and accessible in any sample type, regardless of thickness or preparation. Our team designs and synthesizes kits for multiplexed, quantitative, high-resolution bioimaging for academic research, drug development, and clinical pathology/diagnostics.

Our technology, Hybridization Chain Reaction (HCR), is a next-generation signal amplification platform that enables 1-step multiplexed, isothermal, enzyme-free labeling of biomolecules. HCR RNA fluorescence in situ hybridization (FISH) enables researchers to visualize expression of multiple RNA targets simultaneously in the anatomical context of intact tissues. We have also recently extended the benefits of HCR-based amplification to immunohistochemistry (IHC), allowing for accurate and precise protein quantitation with subcellular resolution.

HCR RNA-FISH and HCR IHC products enable simultaneous labeling of up to 10 RNA and protein targets using a single, unified amplification platform. This results in a method that is simpler, more scalable, and superior to any alternative available today.

**Key Features:**

**Straightforward Multiplexing**

HCR enables 1-step parallel signal amplification of all targets simultaneously. Users can multiplex up to 10 targets! B6 – B10 amplifier channels are now available to order as custom amplifiers. If interested, please specify the amplifier channel you'd like in the comments field on the ordering interface.

**No Custom Design Fees**

We know that custom fees can deter researchers from purchasing key research tools – that's why we don't charge any custom design fees to make our technology accessible to scientists around the world! One of our core values at MI is supporting our users in pursuing fundamental and groundbreaking research, so we design custom probes for any target RNA at no additional cost.

**Deep Sample Penetration**

Existing in situ hybridization techniques either require extensive sample pretreatments to facilitate probe diffusion into thick tissue samples or cannot accommodate thick sample types at all. In contrast, HCR reagents are engineered to be small so that they can deeply penetrate into all sample types including thick tissue and whole-mount samples.

**Looking to try HCR? Scan this QR code to register for access to a free HCR RNA-FISH Starter Kit:**

Each HCR RNA-FISH Starter Kit will come with everything you need to conduct a 3-plex HCR RNA-FISH experiment: 3 probe sets, 3 HCR amplifiers, and a set of buffers. Each kit contains carefully selected targets that have been validated either by our expert HCR users or by the MI team.

## Silver Sponsors


Gene Tools manufactures Morpholino oligos for blocking translation, modifying splicing or inhibiting miRNA activity. Morpholinos are effective, specific, stable and non-toxic. They are used in cell cultures, embryos or, as Vivo-Morpholinos, in adult animals. Gene Tools also markets products for delivery of Morpholinos into cell cultures, including our Endo-Porter endosomal release agent. Dr. Jim Summerton founded the pioneering antisense company Antivirals Inc. (now AVI BioPharma Inc.) in 1980 to develop antisense therapeutics and founded Gene Tools, LLC in 1997 to supply Morpholino oligos to researchers worldwide. Backed by Ph.D.-level customer support, Gene Tools designs and synthesizes Morpholinos and delivery reagents.

THE UNIVERSITY OF CHICAGO MARINE BIOLOGICAL LABORATORY

# PROGRAM AT A GLANCE

| Time | Thursday, Oct 6 | Friday, Oct 7 | Saturday, Oct 8 | Sunday, Oct 9 | Monday, Oct 10 |
|---|---|---|---|---|---|
| 7:00 – 8:30 | Registration/Breakfast | Registration/Breakfast | Breakfast | Breakfast | Breakfast |
| 8:30 – 9:00 | | Session 1: *How to Make a Research Organism* | Session 5: *Developmental Disorders* | Session 9: *Neural Circuits & Diseases* | |
| 9:00 – 9:30 | **Genomics Workshop** Session A: *Aquatic Genomic Resources* | | | | Departure |
| 9:30 – 10:00 | | | | | |
| 10:00 – 10:30 | Coffee Break | Coffee Break | Coffee Break | Coffee Break | |
| 10:30 – 11:00 | Session A: *Aquatic Genomic Resources* | Session 2: *Emerging Model Systems* | Session 6: *Cancer & Aging* | Session 10: *Aquatic Skeletons* | |
| 11:00 – 11:30 | | | | | |
| 11:30 – 12:00 | Sponsor Info Session | | | | |
| 12:00 – 12:30 | Lunch | Lunch | Lunch<br>Aquatics Nutrition Workshop | Lunch | |
| 12:30 – 1:00 | | | | | |
| 1:00 – 1:30 | | | | | |
| 1:30 – 2:00 | Session B: *Functional Investigation of Aquatic Genomes* | Session 3: *Regeneration I* | Session 7: *Immunology & Microbiome* | Session 11: *Genome Engineering in Aquatic Models* | |
| 2:00 – 2:30 | | | | | |
| 2:30 – 3:00 | | | | | |
| 3:00 – 3:30 | Coffee Break | Coffee Break | Coffee Break | Coffee Break | |
| 3:30 – 4:00 | Session C: *Translating Aquatic Genomes to Human Disease* | Session 4: *Regeneration II* | Session 8: *Toxicology & Environmental Health* | Session 12: *Personalized Medicine & Therapeutics* | |
| 4:00 – 4:30 | | | | | |
| 4:30 – 5:00 | | | | | |
| 5:00 – 5:30 | Community Discussion | NIH Peer Review Info Session | Germplasm Cryopreservation Workshop | AQMHD Business Meeting | |
| 5:30 – 6:00 | **AQMHD2022** Registration – Welcome | | | | |
| 6:00 – 6:30 | | Dinner | Dinner | Keynote: *John Postlethwait* | |
| 6:30 – 7:00 | Keynote: *Erich Jarvis* | | | | |
| 7:00 – 7:30 | | Keynote: *Kenneth Poss* | Keynote: *Kristine Willet* | | |
| 7:30 – 8:00 | | | | Conference Lobster Dinner<br>Awards Ceremony | |
| 8:00 – 8:30 | Opening Dinner Reception | | | | |
| 8:30 – 9:00 | | Lightning Talks Poster Session 1 | Lightning Talks Poster Session 2 | | |
| 9:00 – 9:30 | | | | | |
| 9:30 – 10:00 | | | | | |

# SCIENTIFIC PROGRAM

| Time | Workshop: Genomic Tools for Aquatic Genomes<br>Thursday, October 6th | Location |
|---|---|---|
| 7:00-9:00 AM | Registration and Breakfast | Swope |
| 9:00-9:15 | Welcome | Lillie |
| 9:15-11:30 | **Session A. Genomic Resources for Aquatic Models**<br><br>Chair: Matthew Harris (Boston Children's Hospital & Harvard Medical School) | Lillie |
| 9:15-9:45 | **Wes Warren** (University of Missouri)<br>*Aquatic genome resources and their trait discovery applications* | |
| 9:45-10:15 | **Caroline Albertin** (MBL)<br>*How to build a cephalopod: insights from the genome* | |
| 10:15-10:30 | Coffee Break | Loeb Tent |
| 10:30-11:00 | **Stephen Treaster** (Harvard Medical School)<br>*Genomics of exceptional longevity and its variation in the ocean quahog,* Arctica islandica | Lillie |
| 11:00-11:30 | **Kang Du** (Texas State University)<br>*Genome resources and reticulate phylogenomics of* Xiphophorus | |
| 11:30-12:00 | **Workshop Sponsor Information**<br>**Mark Daly** (Cantata Bio)<br>*Haplotype-resolved de novo genome assemblies of non-model organisms* | |
| 12:00-1:30 PM | Lunch | Swope |
| 1:30-3:00 | **Session B. Functional Investigation of Aquatic Genomes**<br><br>Chair: Shawn Burgess (NHGRI) | Lillie |
| 1:30-2:00 | **Jeffrey Farrell** (NICHD)<br>*Cell states and cell fates during zebrafish development* | |
| 2:00-2:30 | **Gert Jan Veenstra** (Radboud University, Netherlands)<br>*Epigenomic interrogation of embryonic development* | |

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| 2:30-3:00 | **Jeramiah Smith** (University of Kentucky)<br>*Cyclostome genomes: Changes in form and function over eons and across development* | |
| 3:00-3:30 | Coffee Break | Loeb Tent |
| 3:30-5:00 | **Session C. Translating Aquatic Genomes to Human Disease**<br><br>Chair: Jeffrey Farrell (NICHD) | Lillie |
| 3:30-4:00 | **Christian Mosimann** (CU Anschutz)<br>*Enhancer discovery, testing, and cross-species comparisons using zebrafish* | |
| 4:00-4:30 | **Jacob Musser** (Yale University)<br>*Sponges as a model for the origin and evolution of animal cell types* | |
| 4:30-5:00 | **Arjun Krishnan** (CU Anschutz)<br>*Computational approaches for translating data and knowledge across species* | |
| 5:00-5:30 | **Community Discussion: Future of Aquatic Genomics for Human Disease**<br><br>Moderator: Ingo Braasch (Michigan State University) | |

| Time | AQMHD 2022<br>Thursday, October 6th | Location |
|---|---|---|
| 2:00-6:00 PM | Registration | Swope |
| 6:15-6:30 | Welcome by the Co-organizers | Lillie |
| 6:30-8:00 | **Keynote Lecture: Erich Jarvis** (The Rockefeller University)<br>*The Vertebrate Genomes Project: Sequencing life for a new era in biology*<br><br>Introduction: Patricia Schneider (Louisiana State University) | |
| 8:00-10:00 | Opening Dinner Reception | Swope Tents |

## Friday, October 7th

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|---|---|---|
| 7:00-8:30 AM | Registration and Breakfast | Swope |
| 8:30-10:00 | **Session 1. How to Take a Research Organism**<br><br>Chair: Mike Schmale (U Miami) | Lillie |
| 8:30-9:00 | **Manfred Schartl** (Texas State University)<br>Xiphophorus*, an evolutionary model for epistatic interactions in disease* | |
| 9:00-9:30 | **Veronica Hinman** (Carnegie Mellon University)<br>*Making a model organism knowledgebase: Echinobase.org as a case study* | |
| 9:30-9:45 | **Nicolas Rohner** (Stowers Institute)<br>*Regulatory networks underlying metabolic adaptation and physiological resilience in animals* | |
| 9:45-10:00 | **Maria Teresa Gutierrez-Wing** (Louisiana State University)<br>*Development of a network to safeguard aquatic biomedical models* | |
| 10:00-10:30 | Coffee Break | Loeb Tent |
| 10:30-12:00 | **Session 2. Emerging Model Systems**<br><br>Chair: Nicolas Rohner (Stowers Institute) | Lillie |
| 10:30-11:00 | **Andrew Gillis** (MBL)<br>*Adult chondrogenesis and spontaneous cartilage repair in the skate (*Leucoraja erinacea*)* | |
| 11:00-11:30 | **Chiara Anselmi** (Stanford University)<br>Botryllus schlosseri*, an emerging evo-devo model for the study of neurogenesis, neurodegeneration, and aging* | |
| 11:30-11:45 | **Jonathan Henry** (MBL)<br>*Automated aquatic systems and the development of marine model systems* | |
| 11:45-12:00 | **Kate Castellano** (Gloucester Marine Genomics Institute)<br>*Molecular and cell culture tools to advance sea urchins as models for aging and cancer resistance* | |

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| 12:00-12:15 | **Javier Rodriguez-Casariego** (Florida International Univ.)<br>*Behavioral and molecular responses to hypoxia in* Aplysia californica | |
| 12:15-1:45 PM | Lunch | Swope |
| 1:45-3:00 | **Session 3. Stem Cells & Regeneration I**<br>(joint with MBL SCARE Course)<br><br>Chair: Jennifer Morgan (MBL) | Lillie |
| 1:45-2:15 | **Shawn Burgess** (National Human Genome Research Institute)<br>*A regulatory network of Sox and Six transcription factors initiate a cell fate transformation during hearing regeneration in adult zebrafish* | |
| 2:15-2:45 | **Tyler Square** (UC Berkeley)<br>*Genes underlying human Ectodermal Dysplasias control tooth localization and regeneration in teleost fishes* | |
| 2:45-3:00 | **Noam Hendin** (Tel Aviv University, Israel)<br>*Molecular characterization of the immediate wound response of the regenerative solitary ascidian* Polycarpa mytiligera | |
| 3:00-3:30 | Coffee Break | Loeb Tent |
| 3:30-5:00 | **Session 4. Stem Cells & Regeneration II**<br>(joint with MBL SCARE Course)<br><br>Chair: Randall Voss (University of Kentucky) | Lillie |
| 3:30-4:00 | **Sven Reischauer** (Justus-Liebig University Giessen, Germany)<br>*Interleukin-11 signaling orchestrates heart regeneration by promoting regenerative reprogramming and limiting scar formation* | |
| 4:00-4:30 | **Igor Schneider** (Louisiana State University)<br>*Deep evolutionary origin of limb regeneration* | |
| 4:30-4:45 | **Timothy Duerr** (Northeastern University)<br>*Retinoic acid breakdown is required for positional identity along the proximodistal axis of the regenerating axolotl limb* | |
| 4:45-5:00 | Break | |
| 5:00-6:0 | **Info Session: Navigating NIH Peer Review**<br><br>**Zubaida Saifudeen** (NIH Scientific Review Officer) | Lillie |

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| 6:00-7:00 | Dinner | Swope |
| 7:00-8:30 | **Keynote Lecture: Kenneth Poss** (Duke University)<br>*Regulating tissue regeneration*<br><br>Introduction: Catherine McCusker (University of Massachusetts Boston) | Lillie |
| 8:30-10:00 | **Lightning Talks & Poster Session 1 with Mixer**<br>(joint with MBL SCARE Course) | Meigs Room/ Swope Tents |

**Saturday, October 8th**

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|---|---|---|
| 7:00-8:30 AM | Breakfast | Swope |
| 8:30-10:00 | **Session 5. Developmental Disorders**<br><br>Chair: Ben Lovely (University of Louisville) | Lillie |
| 8:30-9:00 | **Crystal Rogers** (UC Davis)<br>*Naproxen exposure during early development inhibits cranial chondrogenesis in axolotl embryos* | |
| 9:00-9:30 | **Christian Mosimann** (CU Anschutz)<br>*Lateral thinking in syndromic congenital disease* | |
| 9:30-9:45 | **Brittney Voigt** (UT Austin)<br>*Novel regulators of notochord development suggest a role for purinergic signaling in thoracic spine morphology* | |
| 9:45-10:00 | **Ben Lovely** (University of Louisville)<br>*Genetic screening for ethanol-sensitive zebrafish mutants identifies complex signaling interactions in anterior endoderm and jaw development* | |
| 10:00-10:30 | Coffee Break | Loeb Tent |
| 10:30-12:00 | **Session 6. Cancer & Aging**<br><br>Chair: Peggy Biga (University of Alabama) | Lillie |
| 10:30-11:00 | **Brigitte Galliot** (University of Geneva, Switzerland)<br>*Key role of autophagy in sustained regeneration and slow aging* | |

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| 11:00-11:30 | **Yuan Lu** (Texas State University)<br>*Oncogenic allelic interaction in* Xiphophorus *highlights hybrid incompatibility* | |
| 11:30-11:45 | **Elizabeth Schabot** (University of Miami)<br>*High mutation levels observed in mitochondrial genomes of damselfish neurofibromatosis tumors* | |
| 11:45-12:00 | **Peggy Biga** (University of Alabama)<br>Xiphophorus *fishes as models for understanding sex difference in aging* | |
| 12:00-1:30 PM | Lunch | Swope |
| 12:30-1:30 PM | **Workshop: Aquatics Nutrition and Reference Diet Development** | Lillie |
| 12:30-12:40 | **Steve Watts** (University of Alabama)<br>*Introduction to nutrition and standard reference diet* | |
| 12:40-12:50 | **Zoltan M. Varga** (Zebrafish International Resource Center)<br>*Study overview and reference standard diet testing at the ZIRC* | |
| 12:50-1:00 PM | **Yuan Lu** (Xiphophorus Genetic Stock Center)<br>*Assessment of various standard fish diets on growth, fecundity, and microbiome of* Xiphophorus maculatus *(platyfish) and* Oryzias latipes *(medaka)* | |
| 1:00-1:10 | **Mike Kent** (Oregon State University)<br>*Impact of diet on growth and disease susceptibility in zebrafish* | |
| 1:10-1:20 | **Tom Sharpton** (Oregon State University)<br>*The impact of diet on the zebrafish gut microbiome* | |
| 1:20-1:30 | **Discussion:** *Community Feedback and Community Needs - Nutrition and Development of Standard Reference Diets* | |
| 1:30-3:00 | **Session 7. Immunology & Microbiome**<br><br>Chair: Frauke Seemann (Texas A&M-Corpus Christi) | Lillie |
| 1:30-2:00 | **Chris Amemiya** (UC Merced)<br>*Convergence, divergence, and cooption in the adaptive immune system in the "other" major vertebrate lineage* | |

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| 2:00-2:30 | **Lihua Ye** (Ohio State University)<br>*An enteroendocrine-vagal sensory pathway that transmit gut bacterial signal to the brain* | |
| 2:30-2:45 | **Thomas Sharpton** (Oregon State University)<br>*Using zebrafish to disentangle the impact of environmental exposure on host-microbiome interactions* | |
| 2:45-3:00 | **Masato Yoshizawa** (University of Hawaii)<br>*Social-like behavior is still inducible in the evolutionarily asocial cavefish by a dietary intervention* | |
| 3:00-3:30 | Coffee Break | Loeb Tent |
| 3:30-5:00 | **Session 8. Toxicology & Environmental Influences on Health**<br><br>Chair: Mark Hahn (Woods Hole Oceanographic Institute) | Lillie |
| 3:30-4:00 | **Ramon Lavado** (Baylor University)<br>*The role of altered biotransformation pathways in the rapid adaptation of Gulf killifish to legacy pollutants* | |
| 4:00-4:30 | **Jacob Daane** (University of Houston)<br>*Déjà vu: exploring 'replicate' radiations of perciform fishes to understand the genetic and developmental origins of key traits and adaptation to extreme environments* | |
| 4:30-4:45 | **Khai Ang** (Penn State University)<br>*Web-based reference atlas of* Daphnia magna | |
| 4:45-5:00 | **Frauke Seemann** (Texas A&M-Corpus Christi)<br>*Ancestral benzo[a]pyrene exposure as a risk factor for reduced bone mineralization* | |
| 5:15-6:15 | **Workshop: Germplasm Cryopreservation & Open Hardware**<br><br>Organizers: Yue Liu & Terrence Tiersch (Louisiana State University) | Meigs Room |
| 5:15-5:25 | **Terrence Tiersch** (Louisiana State University)<br>*Germplasm Repositories and Community Development for Genetic Resources of Aquatic Species: A Role for Open Technologies* | |

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| 5:25-6:05 | **Jack Koch, Lucia Arregui, Maria Teresa Gutierrez-Wing** (Louisiana State University)<br>*Open Hardware Demonstrations* | |
| 6:05-6:15 | **Yue Liu** (Louisiana State University)<br>*An Open-hardware Movement to Support Development of Germplasm Repositories for Aquatic Biomedical Models: Conclusion and Quiz with Awards* | |
| 6:15-7:15 | Dinner | Swope |
| 7:15-8:45 | **Keynote Lecture: Kristine Willet** (University of Mississippi)<br>*Sex-dependent, epigenetic, and multigenerational adverse outcomes in zebrafish following parental benzo[a]pyrene exposure*<br><br>Introduction: Frauke Seemann (Texas A&M -Corpus Christi) | Lillie |
| 8:45-10:00 | **Lightning Talks & Poster Session 2 with Mixer** | Meigs Room/ Swope Tents |

**Sunday, October 9th**

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|---|---|---|
| 7:00-8:30 AM | Breakfast | Swope |
| 8:30-10:00 | **Session 9. Neural Circuits & Diseases**<br>Chair: Lynne Fieber (University of Miami) | Lillie |
| 8:30-9:00 | **Deirdre Lyons** (UC San Diego, Scripps Institute)<br>*The nudibranch* Berghia stephanieae *is a new experimental system for studying molluscan brain development* | |
| 9:00-9:30 | **Alberto Stolfi** (Georgia Institute of Technology)<br>*Regulation of neuron and muscle sub-type traits in the non-vertebrate marine chordate* Ciona | |
| 9:30-9:45 | **Erik Duboue** (Florida Atlantic University)<br>*Whole brain imaging in the blind cavefish reveals anatomical and functional evolution of a precise neural circuit modulating light responsiveness* | |

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| 9:45-10:00 | **Nicholas Kron** (University of Miami)<br>*Aplysia californica as a model for viral infection in neurological aging* | |
| 10:00-10:30 | Coffee Break | Loeb Tent |
| 10:30-12:00 | **Session 10. Aquatic Skeletons & Application to Human Disorders**<br><br>Chair: Christoph Winkler (National University of Singapore) | Lillie |
| 10:30-11:00 | **Ryan Gray** (UT Austin)<br>*Common threads of spine morphogenesis in zebrafish* | |
| 11:00-11:30 | **Chrissy Hammond** (University of Bristol, UK)<br>*Skeletal repair and regeneration in ageing zebrafish* | |
| 11:30-11:45 | **Elizabeth Bearce** (University of Oregon)<br>*Urotensin-II-related peptides, Urp1 and Urp2, control zebrafish spine morphology* | |
| 11:45-12:00 | **Christoph Winkler** (National University of Singapore)<br>*From tank to bedside: Transcriptome profiling in a medaka osteoporosis model identifies a novel blood marker that reliably predicts risk for osteoporotic hip fractures in men* | |
| 12:00-1:30 PM | Lunch | Swope |
| 1:30-3:00 | **Session 11. Genome Engineering in Aquatic Models**<br><br>Chair: Marko Horb (National Xenopus Resource Center) | Lillie |
| 1:30-2:00 | **Scott Juntti** (University of Maryland)<br>*Dissecting the regulation of social behaviors in cichlid fishes using gene editing tools* | |
| 2:00-2:30 | **Amro Hamdoun** (UC San Diego, Scripps Institute)<br>*Foundational building blocks for stable genetic modification of sea urchins* | |
| 2:30-2:45 | **Johanna Kowalko** (Lehigh University)<br>*Genetic and developmental factors contributing to eye development and degeneration in* Astyanax mexicanus | |

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| 2:45-:300 | **Sian Martin** (European Xenopus Resource Centre)<br>*Dr. Frog will see you now: How* Xenopus *are moving back towards medicine by modelling human genetic diseases* | |
| 3:00-3:30 | Coffee Break | Loeb Tent |
| 3:30-5:00 | **Session 12. Personalized Medicine & Therapeutics**<br><br>Chair: Manfred Schartl (Texas State University) | Lillie |
| 3:30-4:00 | **Mandë Holford** (Hunter College/AMNH)<br>*Out of the sea and into the well: Developing models for the evolution of venom in marine mollusks* | |
| 4:00-4:30 | **Annapurna Poduri** (Harvard Medical School)<br>*Zebrafish models of genetic epilepsies - Opportunities for translation* | |
| 4:30-4:45 | **Harini Iyer** (Stanford University)<br>*Lysosomal signaling in microglia and Alzheimer's disease* | |
| 4:45-5:00 | Break | |
| 5:00-6:00 | **AQMHD Business Meeting** | Lillie |
| 6:00-7:30 | **Keynote Lecture: John Postlethwait** (University of Oregon)<br>*Connecting aquatic models to human disease*<br><br>Introduction: Ingo Braasch (Michigan State University) | Lillie |
| 7:30-10:00 | **Conference Dinner & Awards Ceremony** | Swope Tents |

## Monday, October 10th

| | | |
|---|---|---|
| 7:00-9:00 AM | Breakfast | Swope |
| afterwards | Departure (checkout until 10:00AM) | |

# ABSTRACTS

## Workshop: Genomic Tools for Aquatic Models

### Aquatic genome resources and their trait discovery applications

**Wesley C. Warren** (University of Missouri)
Edward S. Rice, Yuan Lu, Alex Keene, Nicholas Rohner, Manfred Schartl

Cross-species hybridization of genomes often reveals parental trait origins. To better understand the polygenic complexity of the parental influenced phenotype among aquatic species, we have created reference genomes using long-read sequencing for species in the three genera *Xiphophorus*, *Astyanax*, and *Poecilia*, each with natural or experimentally induced genome hybridization occurrence. A traditional strength of the *Xiphophorus* model system involves the assessment of genetic inheritance patterns following cross-species hybridization, especially melanoma progression. We have generated highly contiguous assemblies of three *Xiphophorus* species: *X. maculatus*, *X. couchianus* and *X. hellerii*, to study adaptive epistatic interactions which are connected to traits modeling human disease conditions. Despite minor genome synteny differences between these *Xiphophorus* species, we have discovered novel structural variants (SV) predicted to be gene disruptive and unique to each species. In the Amazon molly, *Poecilia formosa*, a natural hybrid species originating from a cross of a female *P. mexicana* with a *P. latipinna* male that reproduces asexually, we are studying the long-term effects of no meiotic recombination. We have assembled the original parental genomes using a phasing technique and experiments are underway to test hypotheses of genome decay. The experimental use of the *Astyanax mexicanus* (Mexican tetra) is rapidly expanding due to its many troglomorphic trait adaptations. With multiple independent adaptations of the surface to cave transition previously identified we have sequenced and assembled a surface and two cave morphs, Tinaja and Molino, to study genome-wide SVs which are likely to be major evolutionary events that preceded trait adaptation. The reference genome is a data model that sits at the center of nearly all genome and transcriptome analysis and these aquatic model genomic resources promise to reveal how each parental chromosome contributes to a phenotype.

### How to build a cephalopod: insights from the genome

**Caroline Albertin** (Marine Biology Laboratory)

Coleoid cephalopods have a suite of evolutionary innovations, including their elaborate, highly centralized nervous systems and camera eyes, which are classic examples of convergent evolution. Coleoids also present a number of true novelties, including their adaptive coloration system and sucker-lined arm crown, which have no obvious correlates in other animals. To study the genetic basis underlying these morphological innovations, we sequenced the genome of the longfin inshore squid *Doryteuthis pealeii*. We find that the *D. pealeii* genome is larger than that of *Octopus bimaculoides*. The substantial increase in C2H2-zinc finger genes observed in the *O. bimaculoides* genome is absent in *D. pealeii*. However, the massive expansion of protocadherins, a family of cell adhesion molecules important for wiring vertebrate brains, described in octopus appears to be even larger in squid. Our chromosome-scale assembly also reveals many local expansions of genes expressed in novel cephalopod structures, including genes involved in iridophore and sucker function. Some of these gene clusters are cephalopod-, or even squid-specific, while others appear to be local duplications of genes found in distantly related animals. These data highlight a major role for the acquisition of novel genes and gene family expansion in the evolution of cephalopod morphological innovations. In addition, these genome assemblies are the foundation for developing CRISPR-mediated gene knockouts to study gene function in cephalopods.

**Genomics of exceptional longevity and its variation in the ocean quahog, *Arctica islandica***

**Stephen Treaster** (Harvard Medical School Genetics, Boston Children's Hospital Orthopedics)
Doris Abele, Christoph Held, Matthew Harris

Longevity can vary widely among individuals in a population as well as several orders of magnitude between species. Although the foundations for this lifespan potential is hereditary, little is known of how longevity is encoded within the genome. To understand these mechanisms, we look to the ocean quahog, *Arctica islandica*, which can survive for over five-hundred years off the coast of Iceland. However, a thousand miles away near Helgoland in the German Bight, the same species survives for only two-hundred years, while those extending into the Baltic Sea attain only thirty-five years of age. To unlock the secrets underlying this broad regulation of lifespan, we generated a chromosome-level, high-quality reference genome for Arctica islandica and sequenced individuals from each of these populations. We annotated transcriptional profiles of several tissues to begin analysis of gene function in this non-model organism. As *Arctica islandica* is the only remaining extant species of the family Arcticidae dating back to the Jurassic Period, the genome provides a valuable phylogenetic reference to understand bivalve genome evolution. The recent population expansion into the Baltic Sea, which formed in the early Holocene as the last ice sheets retreated ~10,000 years ago, provides an opportunity to specifically address variation of lifespan within this species. The dramatic reduction in maximum lifespan between the populations may inform novel genetic regulators of longevity as well as life-history strategies that may associate with such changes. Here, we characterize the *Arctica islandica* genome as compared to other Venerid Bivalves and perform population genetics analyses to understand the genetics of extreme longevity.

**Genome resources and reticulate phylogenomics of *Xiphophorus***

**Kang Du** (Xiphophorus Genetic Stock Center, Texas State University)
Yuan Lu, Molly Schumer, Hyun Park, Axel Meyer, Manfred Schartl

Negative epistasis plays an essential role in human disease etiology. Such incompatible gene interactions are often between diverged alleles. The profound phenotypical and genetic divergence between *Xiphophorus* species, and their capacity to produce viable interspecies hybrids render this small fish genus an unique model system for disease-causing and modifying gene discovery. For example, genes that drive and modulate melanoma were identified using the *Xiphophorus* interspecies hybrid system (*xmrk*, *rab3d*, and *adgre5*). To facilitate the disease controlling and modulating gene and genetic pathway discovery, advanced genomic resources are crucial. Herein we assembled the genomes of all the *Xiphophorus* species, reconstructed the reticulate phylogeny, and revealed the evolution of some genetic combinations that potentially decrease fitness of hybrids by inducing diseases.

**Haplotype-resolved de novo genome assemblies of non-model organisms**

**Mark Daly** (Cantata Bio)

Traditional de novo genome assembly workflows produce a pseudo-haploid assembly. This is a mosaic of the two haplotypes and does not represent a true diploid genome. We will present our latest workflow for building true diploid and polyploid assemblies, where a genome assembly is produced for each haplotype. The technology relies on highly accurate PacBio HiFi data, and the very even, whole-genome coverage of Omni-C proximity ligation data.

### Cell states and cell fates during zebrafish development

**Jeffrey Farrell** (NICHD)
Abhinav Sur, Yiqun Wang, Paulina Capar, Gennady Margolin, Cheng-Yi Chen, Eric Upton, Jeremy Popowitz

Animals consist of a collection of cells with beautifully diverse shapes, structures, and functions, and this diversity is rebuilt from scratch in every embryo. Single-cell RNAseq approaches provide the possibility of mapping the molecularly distinct populations of cells during development and inferring the changes in gene expression that occur as those cells acquire specific identities, functions, and morphologies. To investigate a broad range of cell types undergoing differentiation during wild-type development, we sequenced ~450,000 cells from closely spaced stages spanning late zebrafish embryogenesis and early larval development (14-120 hours post-fertilization) and integrated that with our published data from 3-12 hours post-fertilization. Using these data, we searched for gene expression programs that are shared between multiple cell types, creating a catalog of gene expression programs that are re-used during development to create shared cellular features. Additionally, we generated high-resolution cell atlases of individual tissues and reconstructed the developmental trajectories of gene expression changes leading to distinct cell types. This has enabled categorization of cell types in tissues with poor molecular definitions (such as smooth muscle and pericyte cells) and revealed unknown or poorly characterized molecular cell states in many tissues. For instance, we find an uncharacterized population of surfactant producing cells, as well as a small population of best4+/otop2+ intestinal enterocytes, which we propose are homologous to a recently discovered human intestinal cell type that is depleted in inflamed colons. The origin of best4+/otop2+ enterocytes and the signals and transcription factors that specify them remain unknown; our approach suggests the transcription factors that may define these cells, which we are testing using crispant approaches.

### Epigenomic interrogation of embryonic development

**Gert Jan Veenstra** (Radboud University, Whitman Center, Marine Biological Laboratory)
Saskia Heffener, Rebecca Snabel

Histone modifications and their regulation play important roles in embryonic development. Secreted factors constitute major inductive signals, affecting many aspects of cellular physiology including transduction of the signals to the nucleus. Within the nucleus, transcription factors activate target genes within the constraints of chromatin, the state of which is thought to both reflect developmental history and cellular potency. How epigenetic mechanisms contribute to cell lineage commitment, however, is not well understood.
Exploitation of the available genome sequence for molecular and functional analysis of developmental mechanisms requires a well-annotated genome. This involves assembling an inventory of all expressed sequences and their linkage in transcription units, in addition to identification of relevant cis-regulatory elements.
We are profiling the genome-wide distribution of histone modifications and are integrating this information with bulk and single nucleus RNA-sequencing data, for the analysis of heart development in vertebrates (lamprey, frog) and cephalopods (inshore squid), in collaboration with Marko Horb, Karen Crawford, Caroline Albertin, Jennifer Morgan and Joshua Rosenthal (MBL). This approach uncovers epigenomic and gene-regulatory mechanisms of development and their modifications during evolution, while also improving genomic annotation.

**Cyclostome genomes: Changes in form and function over eons and across development**

**Jeramiah Smith** (University of Kentucky)
Nataliya Timoshevskaya, Kaan Eşkut

Lampreys and hagfish are living representatives of a lineage (Agnatha, Cyclostomata: jawless vertebrates) that diverged from the common ancestor of all other living vertebrates (Gnathostomata: jawed vertebrates) more than 500 million years ago. Advances in sequencing and assembly methods are allowing the development of accurate chromosome-scale assemblies for an increasing number of cyclostome genomes. These assemblies improve our ability to resolve ancient evolutionary events that define the gene content and chromosomal structure of living vertebrate taxa.
Additionally, both lamprey and hagfish possess an exceptional mode of gene silencing, wherein specific portions of the genome are removed from most cell lineages during early development and retained only in the germline (known as programmed DNA loss). These germline-specific regions present particularly challenging targets for genome assembly due to an enrichment of repeats and other complex sequence structures. Improved assemblies of these regions have revealed roles of ancient and recent segmental duplication from somatically retained chromosomes, intrachromosomal duplication and adaptive evolution of in shaping the content and function of eliminated sequences.
Subcellular structures that are formed during programmed DNA loss mimic features associated catastrophic DNA damage in human but are tightly regulated as a feature of normal development in sea lamprey. The ability to produce large numbers of synchronously developing embryos by in vitro fertilization has facilitated studies of the mechanisms of DNA loss. Chemical and Cas9 knockout screens implicate components of small/noncoding RNA processing, centriolar/ciliary components, nuclear membrane dynamics, and canonical epigenetic silencing pathways as functionally relevant to programmed elimination. These ongoing studies are also guiding our efforts to more broadly reconstruct the evolution of vertebrate genomes and early embryonic reprogramming.

**Enhancer discovery, testing, and cross-species comparisons using zebrafish**

**Christian Mosimann** (University of Colorado School of Medicine, Anschutz Medical Campus)

Enhancers act as gene-regulatory elements spread throughout the genome, controlling cell-type specific gene expression through the binding of tissue- or signaling-specific transcription factors. Major challenges in discovering and validating enhancer elements remain the lack of concise predictive criteria across cell types and model systems and of widely accessible, streamlined methods to test enhancer activity in vivo. In particular, the functional validation of enhancers with human disease-associated variants remains challenging.
To streamline regulatory element testing in zebrafish, we built a suite of Multisite Gateway-compatible vectors for Tol2-based transgenesis. We document mCerulean as suitable fluorophore to expand the standard spectrum of fluorescent reporter and the small (less than 130 bp) mouse beta-globin minimal promoter as versatile enhancer testing tool in zebrafish and additional species. Further, we introduce pineal gland-based transgenesis markers for quality control and combinatorial transgene experiments.
Applying these tools, we functionally tested and defined early mesoderm and endoderm enhancers bound by EomesA, FoxH1, and Mixl1 (EFM) that act as minimal input for lateral plate mesoderm induction. We mapped the regions jointly occupied by EFM using available ChIP-seq data and applied mCerulean-based reporter transgene tools to validate enhancer candidates in transient and stable zebrafish transgenics. Our analysis revealed EFM-responsive gene-regulatory elements in several mesoderm and endoderm genes at the base of classic ventro-lateral expression patterns in zebrafish. Our new transgenic reporters establish in vivo readouts for genotype-phenotype testing and cell fate sensors to refine reprogramming approaches towards therapeutic cell types.

**Sponges as a model for the origin and evolution of animal cell types**

**Jacob, Musser** (Yale University)
Detlev, Arendt, Leonid Moroz

The origin of animal multicellularity is one of the great mysteries of animal evolution, requiring the evolution of distinct cell types to coordinate complex multicellular behavior.
Sponges are an ancient animal lineage with only a handful of cell types and without a nervous system or muscles, making them ideal for early origins of animal cell diversity.
Recently, we pioneered whole-body single-cell RNA sequencing in the freshwater demosponge *Spongilla lacustris* and comprehensively profiled its cell types.
Remarkably, we show sponges possess a functional repertoire of cells similar to that of other animals. These include nitric oxide-sensitive contractile cells, amoeboid phagocytes, and secretory neuroid cells that reside in close contact with digestive cells expressing scaffolding and receptor proteins. Zooming in on neuroid cells using correlative X-ray and electron microscopy reveals secretory vesicles and cellular projections enwrapping choanocyte microvilli and cilia. Our data reveal a communication system organized around sponge digestive chambers, utilizing conserved modules that became incorporated into the pre- and postsynapse in the nervous systems of other animals. These findings place sponges center stage for understanding the origin of multicellularity and complex tissues including the nervous system.

**Computational approaches for translating data and knowledge across species**

**Arjun Krishnan** (University of Colorado Anschutz Medical Campus)
Hao Yuan, Christopher Mancuso, Kayla Johnson, Ingo Braasch

Finding the right research organism, experimental settings, and genes ideal for investigating a given human trait or disease is a fundamental challenge in translation. We are working to addressing this challenge by developing computational approaches that can leverage two powerful sources of publicly available data from humans and research organisms: i) hundreds of thousands of published transcriptomic profiles and ii) genome-scale gene interaction networks. First, we are developing a regression approach to identify transcriptomes in a research organism that can recapitulate a query human transcriptome. Once trained, the regression model can be interpreted to find tissues, experimental conditions, and phenotypes that could bring about a global transcriptional response in the research organism that is equivalent to the human profile. Further, the regression model can be used to predict human-organism genes that have equivalent functions in the biological context captured by the human sample. Second, we are developing an approach to jointly represent gene interaction networks from multiple species in a shared ‘functional space’ where genes in different species that have similar network partners will be close to each other. We are then using network-based machine learning (ML) with these multi-species gene representations as features to predict novel/under-characterized genes in one species that are functionally similar to sets of known genes of interest in another. Finally, we are building a method to create a joint representation of transcriptome profiles from multiple species in a common space based on gene homology information. Then, we are using ML to predict transcriptomes in one species that have patterns of gene expression distinctive to a cell type, phenotype, disease, or drug of interest in another species. These complementary approaches are general and can be applied to map genes, samples, and experimental factors between humans and any research organism.

# AQMHD 2022

## Keynote Lecture

### The Vertebrate Genomes Project: Sequencing Life for a New Era in Biology

**Erich Jarvis**
The Rockefeller University, Howard Hughes Medical Institute

Understanding the mechanisms and evolution of complex traits often requires studying the underlying genes and gene networks. However, trying to decipher the genetic networks responsible for the function, evolution, and mechanisms of these traits requires high-quality genomic data. For this reason and others, a large-scale international project has formed, the Vertebrate Genomes Project (VGP). The mission of the VGP is to produce high-quality, near complete and error-free genomes, for Earth's all ~70,000 vertebrate species, and to use these genomes to address fundamental questions in biology, disease, and conservation. The VGP has become a model for other sister consortiums, such as the Earth Biogenomes Project (EBP), Bat 1K project, Darwin Tree of Life Project (DToL), Africa Biogenomes Project (AfricaBP), among others. Here I will present the progress we have made in generating these genomes, including on genome sequencing and assembly. I will also present benefits that have been made in several key studies on mechanisms and evolution of complex traits. These include the genetics of vocal learning and spoken-language, evolution of the oxytocin gene family, and conservation of critically endangered species. This has allowed us to discover thousands of new regulatory regions in genomes, which were missing in prior genome assemblies. The lessons learned are relevant to all individual and large-scale genomic projects and are expected to bring in a new era in biology.

**About**
Erich Jarvis is a professor at the Rockefeller University. Dr. Jarvis uses song-learning birds and other species as models to study the molecular and genetic mechanisms that underlie vocal learning, including how humans learn spoken language. He is interested in how their brains, and ours, have evolved to produce this complex behavior. Unlike songbirds, the vast majority of animals - including common model organisms like mice and fruit flies - either cannot imitate novel sounds or have limited vocal flexibility, limiting their usefulness in the study of spoken language. To advance research in this field, the Jarvis lab has developed a suite of experimental tools for songbirds and other species to probe the genetics underlying vocal learning. By combining behavioral, anatomical, electrophysiological, and molecular biological techniques, Jarvis hopes to advance knowledge of the neural mechanisms of vocal learning and, more broadly, gain a deeper understanding of how the brain generates, perceives, and learns complex behaviors.
Beyond his work with songbirds, Jarvis uses genomics to understand how vocal-learning and vocal non-learning species are related, providing insight into how vocal learning and other complex behaviors have evolved.
In 2002, the National Science Foundation awarded Dr. Jarvis its highest honor for a young researcher, the Alan T. Waterman Award. In 2005 he was awarded the National Institutes of Health Director's Pioneer Award providing funding for five years to researchers pursuing innovative approaches to biomedical research. In 2008 Dr. Jarvis was selected to the prestigious position of Investigator for the Howard Hughes Medical Institute.

### *Xiphophorus*, an evolutionary model for epistatic interactions in disease

**Manfred Schartl** (Texas State University)
Yuan Lu

*Xiphophorus* species are representatives of the “evolutionary mutant models”. These are unique species who develop adaptive phenotypes that are similar to maladaptive human disease. Such disease-like phenotypes are often result of specific evolutionary adaptations to disease-causing mutations or environment. If the type of mutations that are favored by natural selection in wild populations are similar to those that are involved in human pathological processes, we can better understand the complexity of disease processes by exploring adaptations to counteract the deleterious alleles. Evolutionary models offer unique opportunities to detect and study disease driver genes, disease modifiers, epistatic interactions in disease gene and drug targets. The *Xiphophorus* model offers technical advantages as ease of production, availability of inbred lines, genotypic and phenotypic diversity from wild populations, and extensive resources and tools. Three approaches are used in Xiphophorus: (1) precision breeding that combine Mendelian genetics with genomics and in vitro biochemistry, (2) use of genotypic variation and population genomics of natural hybrids, and (3) use of hybrid incompatibilities and epistatic gene interactions to expand the *Xiphophorus* model to a wide variety of diseases. As proof-of-concept for using *Xiphophorus* to progress understanding of human diseases, *Xiphophorus* became one of the oldest animal models for cancer, in particular melanoma. It satisfies face, predictive, and construct validity. It replicates melanoma clinical findings in humans, predicts pathways, and exhibits molecular drivers, pathways, and disease signatures that are largely consistent with human disease. Although best known for its contribution to melanoma research, other conditions affecting human health can be studied in *Xiphophorus*, e.g. metabolic syndrome, photosensitivity, developmental defects, albinism, using available resources and being developed for a broad research community.

### Making a model organism knowledgebase: Echinobase.org as a case study

**Veronica Hinman** (Carnegie Mellon University)
Saoirse Foley, Bradley I Arshinoff, Gregory A Cary, Kamran Karimi, Sergei Agalakov, Francisco Delgado, Vaneet S Lotay, Carolyn J Ku, Troy J Pells, Thomas R Beatman, Cheryl A Telmer, Charles A Ettensohn, Peter D Vize

Many species of echinoderms have been used as model research organisms in biology; in particular, for embryology, gene regulation, immunology, cell biology, and more recently gene regulatory network evolution and regeneration. This talk will focus on the establishment of *Patiria miniata*, the bat sea star, and several other species of echinoderms as genomic era model organisms. In particular, I will discuss how we have built, Echinobase, - the model organism knowledge base for Echinoderms. I will provide a brief historical perspective starting from the first genome assembly of an echinoderm, through to the current version of Echinobase. I will discuss ways in which we have engaged with the community of echinoderm researchers, established naming conventions, partnered with other model organism databases, namely Xenbase, and built links to other genomic resources such as OMIM, USCS genome browser. PhylomeDB, and the alliance of genome resources. Finally, I present thoughts on how other model organisms can move towards the development of knowledgebases.

**Regulatory networks underlying metabolic adaptation and physiological resilience in animals**

**Nicolas Rohner** (Stowers Institute)

Adapting to extreme environments requires drastic changes to an animal's metabolism. Adaptation to the darkness and food limitation of caves can be challenging. The cavefish *Astyanax mexicanus* is a promising research organism to unravel the genetic basis of starvation resilience. Extant surface and cave morphs of the same species remain interfertile and can be bred outside their natural environments. We previously showed that cavefish evolved impressive adaptations, such as increased appetite, starvation resistance, and altered feeding due to mutations in *mc4r*. In addition, we found that cavefish display elevated blood sugar levels and insulin resistance caused by a mutation in the insulin receptor. In contrast to human patients, carrying the exact same mutation, cavefish do not display common markers of diabetes and live long and healthy lives. Furthermore, cavefish develop hypertrophic visceral adipocytes without signs of inflammation due to reduced amounts of pro-inflammatory cytokines. Taken together, our work suggests that cavefish develop these phenotypes as part of their starvation resistance and have evolved resilience phenotypes that allow them to tolerate stark deviations from what would be considered normal physiology in other vertebrates, including humans. We performed genome-wide epigenetic profiling in the liver tissues of *Astyanax* and found that many of the identified cis-regulatory elements (CREs) have genetically diverged between surface and cave morphotypes, while retaining remarkably similar regulatory signatures between different cave populations. One such CRE in the hpdb gene harbors a genomic deletion in cavefish that abolishes IRF2 repressor binding and derepresses enhancer activity in reporter assays. Selection of this mutation in independent cave populations supports its importance in cave adaptation and provides novel molecular insights into the evolutionary trade-off between loss of pigmentation and adaptation to food-deprived caves.

**Development of a network to safeguard aquatic biomedical models**

**Maria Teresa Gutierrez-Wing** (Louisiana State University AgCenter AGGRC)
Yue Liu, Jack Koch, Lucia Arregui, Terrence R. Tiersch

Aquatic organisms have become powerful models in biomedical research. Tens of thousands of natural, mutant, and transgenic lines are used around the world. Maintaining these genotypes as live animals is expensive, labor intensive, and inherently risky, and cryopreservation has become a necessity. Lack of consistent quality control has resulted in poor reproducibility and reliability. The use of non-standardized protocols can lead to inconsistencies and failures in fertilization, loss of lines, and considerably time, effort and resources waste.
In 2017 an NIH sponsored Cryopreservation Workshop was conducted to develop germplasm repositories to protect aquatic biomedical genetic resources, with the directors of the five NIH-funded aquatic animal stock centers. A mechanism to deliver research and capacity development was identified, consisting of a Hub and repository network, based on the Aquatic Germplasm and Genetic Resources Center (AGGRC, LSU AgCenter), that specifically focus on the translation of research to practice. Expanding on previous work of AGGRC with Zebrafish International Resource Center (ZIRC) and the Xiphophorus Genetic Stock Center (XGSC), efforts to establish comprehensive repository systems in the other three stock centers and establishment of a centralized unit (Hub) at the AGGRC were started with NIH-ORIP funding. The hub will integrate activities across the Ambystoma Genetic Stock Center (AGSC), National Resource for Aplysia (NRA) and National Xenopus Resource (NXR), ZIRC and XGSC and their user communities. The network hub works in close collaboration with the National Animal Germplasm Program (NAGP). Activities such as cryopreservation protocol and pathway development, documentation, quality management, outreach programs, community interaction, freezing services, technology development and training, informed by feedback from the stock centers users, will be available to all members of the network.

**Adult chondrogenesis and spontaneous cartilage repair in the skate (*Leucoraja erinacea*)**

**Andrew Gillis** (Marine Biological Laboratory)

In mammals, cartilage is predominantly an embryonic tissue, forming a transient model of the developing endoskeleton. Most mammalian cartilage is ultimately replaced by bone through the process of endochondral ossification, with cartilage persisting at relatively few sites within the adult skeleton (e.g., in joints, as articular cartilage). Mammalian articular cartilage is an avascular and aneural tissue with very poor capacity for spontaneous repair. Cartilaginous fishes (sharks, skates and rays), on the other hand, possess a skeleton that is composed almost entirely of cartilage, and that remains cartilaginous throughout life. We have found that embryonic development of cartilage in the skate (*Leucoraja erinacea*) closely mirrors that of mammals, with developing chondrocytes co-expressing genes encoding the transcription factors Sox5, Sox6 and Sox9. However, in skate, transcriptional features of developing cartilage persist into adulthood, both in peripheral chondrocytes and in cells of the fibrous perichondrium that ensheaths the skeleton. Using pulse-chase label retention experiments and multiplexed in situ hybridization, we have identified a population of cycling Sox5/6/9+ perichondral progenitor cells that continue to generate new cartilage during adult growth. We also find that persistence of chondrogenesis in adult skates correlates with an ability to spontaneously repair cartilage injuries, with evidence that repair cartilage derives from the perichondrium. We are now using single cell RNA sequencing and comparative genomics to discover transcriptional and genomic correlates of the permanent cartilaginous skeleton of the skate, so that we may use this model for adult chondrogenesis and cartilage repair to guide novel cell-based therapies for articular cartilage injury.

***Botryllus schlosseri*, an emerging evo-devo model for the study of neurogenesis, neurodegeneration, and aging**

**Chiara Anselmi** (Stanford University)
Mark Kowarsky, Irving L Weissman, Ayelet Voskoboynik*, Lucia Manni*
*Equal contribution

Loss of the brain's functional ability is a common symptom of aging and neurodegenerative diseases. While the genetic and molecular mechanisms underlying human neurodegeneration are studied in-depth, very little is known about the evolutionary origin of these traits and their involvement in loss of nervous system function in aged invertebrate species. Here, we characterized the nervous system of *Botryllus schlosseri*, a chordate with a simple central nervous system, as a novel model to study evolutionary neuroscience and neurodegeneration. *B. schlosseri* reproduces both sexually and asexually, with adult brains regenerating and degenerating multiple times throughout its adult life. Combining microscopy, transcriptomics and behavioral assays, we found that each week during colony budding, a decrease in the number of neurons in adult brains is associated with reduced response to stimuli followed by programmed cell death and removal by phagocytes (i.e. 'takeover'). Moreover, we found significant changes in the expression levels of 73 mammalian homologs genes associated with neurodegenerative diseases and neural stem cells pathways during this weekly cycle. Comparing the same weekly cycle in young and old colonies, we found that old colonies contain a significantly lower number of neurons, with changes in 148 unique genes linked to neurodegenerative diseases. Indeed, as a member of an evolutionary clade considered to be a sister group of vertebrates, this organism may be a fundamental resource in understanding how evolution has shaped these processes across phylogeny and obtaining insight on their mechanisms.

**Automated aquatic systems and the development of marine model systems**

**Jonathan Q. Henry** (Marine Biological Laboratory)

Difficulties associated with the supply and culture of live marine animals are major hurdles for biological research, especially for in-land laboratories without direct access to natural, flowing sea water. These animals can be reared in automated, recirculating aquatic systems that incorporate sensors and controls to monitor and adjust water quality, and also automated feeding. By ensuring more optimal culture conditions one can reduce generation time and increase reproductive output to produce large numbers of animals to support lab research. This allows one to rear animals rapidly through successive generations, and ultimately to develop inbred and transgenic lines. Self-contained aquatic stems are critical, even at marine stations, where one maintains non-native species and transgenic lines that cannot be released into the local environment. The speaker has been developing a variety of automated aquatic systems and MBL is now establishing a dedicated facility to house these systems to raise different species to support missions in both teaching and research. These systems are designed to be self-sufficient and run for weeks at a time without human intervention, reducing the challenges of rearing marine animals. As such, these systems save time and effort to improve experimental productivity. A wide variety of species have been successfully reared in these systems, including molluscs, crustaceans, cnidarians, polychaetes, flatworms, etc. In this talk the speaker will describe efforts to develop automated aquatic systems and key lessons learned in the development and care of marine model systems for biological and medical research.

**Molecular and cell culture tools to advance sea urchins as models for aging and cancer resistance**

**Kate Castellano** (Gloucester Marine Genomics Institute)
Jennifer Polinski, Andrea Bodnar

The aging human population is one of the most significant challenges of the 21st century with far-reaching implications for all aspects of society. Sea urchins present a unique opportunity to advance our understanding of aging due to the existence of species with tremendously different natural life spans and exceptional longevity. The red sea urchin (*Mesocentrotus franciscanus*) is one earth's longest living animals, living more than 100 years without showing signs of aging and or cancer. Studies to date, conducted within the framework of known theories of aging, have demonstrated maintenance of telomeres, maintenance of antioxidant and proteasome enzyme activities, and little accumulation of oxidative cellular damage with age. Gene expression studies indicate that key cellular pathways involved in protein homeostasis, tissue regeneration, and neurological function are maintained with age. We recently completed the red sea urchin genome providing a unique opportunity to conduct unbiased genome-wide comparisons of short- and long-lived sea urchin species to uncover new genetic determinants underlying longevity and life-long good health. Genome wide syntenic alignments have revealed sites of chromosome rearrangements that distinguish short- and long-lived species. Gene family expansion and contraction analysis uncovered expanded gene families in long-lived species related to innate immunity, sensory nervous system, and genome stability, including expansion of several tumor suppressor genes. In addition, newly established cell lines from sea urchins now facilitate functional genomic studies that link genotype to phenotype. Given the close genetic relationship between sea urchins and humans, novel insights into mechanisms that promote long-term maintenance of tissue function and healthy aging may ultimately translate to preventative and therapeutic strategies for human age-related degenerative diseases and uncover new avenues for the prevention or treatment of cancer.

**Behavioral and molecular responses to hypoxia in *Aplysia californica***

**Javier Rodriguez-Casariego** (Florida International University)
Lynne Fieber, Mark Miller

Current therapeutic approaches for the treatment of hypoxic/ischemic brain damage can benefit from insights resulting from the study of hypoxia/anoxia resistant organisms. Hypoxia resistance, however, is not a common feature in model organisms like rats and mice, limiting the understanding of neuronal mechanisms of hypoxia resistance and recovery. The California sea hare *Aplysia californica* can be the exception, being naturally exposed to hypoxic or anoxic conditions caused by very dynamic coastal environment they inhabit. However, little is known about the cellular and molecular mechanisms of their resistance or their survival strategy. Here we experimentally exposed two cohorts of A. californica individuals to 6h of hypoxic conditions (< 1.8 mg/ml) in a flow-through water system for 7 days. Time to right (TTR) and Tail withdrawal (TWR) reflexes were evaluated before the exposure, right after reoxygenation, and after 12h recovery for each exposure day. Animals were dissected after 2h, 6h, 6 days, and 7 days of exposure, as well as right after reoxygenation and 12h recovery, and CNS ganglia were sampled for gene expression analysis. Remarkable resistance to hypoxia was observed, with TWR showing lagged and weak responses only after 6 days of repetitive exposure. TTR reflex was never significantly impaired. Preliminary gene expression analyses showed evidence of the early activation of stress response genes, reductions of the cellular metabolism and “frontloading” of genes in anticipation with the hypoxic event after recovery. Overall, here we show the complexity of A. californica response to hypoxia, its remarkable resistance, and its potential to become a critical model for the study of hypoxia in the nervous system.

**A regulatory network of Sox and Six transcription factors initiate a cell fate transformation during hearing regeneration in adult zebrafish**

**Shawn Burgess** (NHGRI)
Erin Jimenez, Claire C. Slevin, Wei Song, Zelin Chen, Stephen C. Frederickson, Derek Gildea, Weiwei Wu, Abdel G. Elkahloun, Ivan Ovcharenko

Using adult zebrafish inner ears as a model for sensorineural regeneration, we ablated the mechanosensory receptors and characterized the single-cell epigenome and transcriptome at consecutive time-points during hair cell regeneration. We utilized deep learning on the regeneration-induced open chromatin sequences and we identified cell-specific transcription factor (TF) motif patterns. Enhancer activity correlated with gene expression and identified potential gene regulatory networks. A pattern of overlapping Sox- and Six-family TF gene expression and binding motifs was detected, suggesting a combinatorial program of TFs driving regeneration and cell identity. Pseudo-time analysis of single-cell transcriptomic data suggested support cells within the sensory epithelium changed cell identity to a “progenitor” cell population that could differentiate into hair cells. We identified a 2.6 kb DNA enhancer upstream of the sox2 promoter that when deleted, showed a dominant phenotype that resulted in a hair cell regeneration-specific deficit in both the lateral line and adult inner ear.

**Genes underlying human ectodermal dysplasias control tooth localization and regeneration in teleost fishes**

**Tyler Square** (UC Berkeley)

Organogenesis is regulated by extracellular signals that govern both organ position and their later regeneration. Some organs, including epithelial appendages like teeth and hair, are organized as fields of iterated and patterned organ units that regenerate. Mutations in single genes in humans, such as Ectodysplasin (Eda), Wnt10a, and Grem2, cause Ectodermal Dysplasia, in which both tooth and hair fields develop abnormally, revealing shared genetic inputs between these disparate epithelial organs. While we know that these genes are required for the normal development of multiple epithelial organ field types, we understand little regarding their sufficiency throughout ontogeny to drive organ field specification and organ regeneration. Using a newly developed set of transgenic stickleback fish and zebrafish that allow temporal control over gene overexpression, we first tested whether Eda and Wnt10a are sufficient to affect tooth field establishment or the regeneration rate of tooth units. In both zebrafish and sticklebacks, upregulation of Eda and Wnt10a contributes to faster tooth regeneration rates, while only Eda is sufficient to expand tooth fields and initiate ectopic teeth. Next, we tested the hypothesis that BMP signaling plays an opposite, inhibitory role on tooth regeneration rate, as occurs during mammalian hair regeneration. In both fish species, upregulation of the BMP inhibitor Grem2a results in grossly similar phenotypes as Wnt10a upregulation, suggesting specific effects on tooth regeneration for these two secreted factors. Conversely, upregulation of Bmp6 or Dkk2, a Wnt pathway inhibitor, reduces tooth regeneration rates and tooth number overall by inhibiting the formation of all new teeth, consistent with the known opposing roles for BMP and Wnt signaling during hair regeneration. Together these data support shared genetic responses for the regeneration of teeth and hair.

**Molecular characterization of the immediate wound response of the regenerative solitary ascidian *Polycarpa mytiligera***

**Noam Hendin** (Tel Aviv University)
Tal Gordon, Noa Shenkar, Omri Wurtzel

The early stages of injury response create the environment that will promote either regeneration or scarring. Yet, analyses of transcriptional changes after injury were performed only on a handful of regenerative organisms. In this study, on the injury response of the highly regenerative ascidian *Polycarpa mytiligera*, an emerging model organism, we used the siphon for studying transcriptional changes following injury in regenerating and non-regenerating tissues and identified a robust gene expression program that is activated at the initial 24 hours post-amputation (hpa). We detected the upregulation of conserved genes, such as BMP1, GHSR and a serine protease inhibitor (PI), expression of which was sustained only in non-regenerating tissue fragments by 24 hpa. We optimized fluorescent in situ hybridization protocol and detected the majority of BMP1+ cells in the ECM-like tunic covering the animal. Analysis of injuries in other body regions suggested BMP1 may be part of a common wound response program. Our results suggest that initially injury-induced programs are similar across organs, however, distinct genetic profiles are observed between the regenerating and the necroting fragments. Ascidians are the closest relatives of vertebrates, making them an important system for studies of regeneration, and *P. mytiligera* is the only known solitary ascidian capable of regenerating every body part. The finding that highly conserved genes, found also in vertebrates, are involved in scar-free injury response highlights the importance of studying diverse regenerating and non-regenerating tissues to regeneration, which is essential for identifying targets for inducing tissue regeneration in non-regenerating animals.

**Interleukin-11 signaling orchestrates heart regeneration by promoting regenerative reprogramming and limiting scar formation**

**Sven Reischauer** (Justus-Liebig-University Giessen, Germany)
Srinivas, Allanki, Boris Strilic, Lilly Scheinberger, Yeszamin Onderwater, Alora Marks, Stefan Günther, Jens Preussner, Khrievono Kikhi, Mario Looso, Didier YR Stainier

Fibrotic scarring is an intrinsic part of the wound repair process across species with the exception of animals that are capable of complete regeneration, including zebrafish. The general opinion is that scarring prevents regeneration and hence is a leading cause of morbidity in humans. However, unifying mechanisms that prevent scarring and promote regeneration remain elusive. Here, employing comparative transcriptional profiling coupled with genetic loss-of-function studies, we identify a single pathway, Interleukin-11 (Il-11)/Stat3 signaling, as a global upstream regulator of regeneration and scarring in zebrafish. We show that animals lacking Il-11 signaling display strongly impaired regeneration across diverse tissues and developmental stages, essentially resembling non-regenerative adult mammals. By analyzing regeneration in the adult heart and fin, we show that Il-11 acts to reprogram stromal cells to activate global and tissue-specific regenerative gene programs, and to broadly limit hallmarks of the adult mammalian scarring response. Using lineage tracing and transgenic approaches, as well as human cells in culture, we show that IL-11 signaling in endothelial cells antagonizes pro-fibrotic transforming growth factor beta (TGF-Î²) signaling and endothelial-to-mesenchymal transition (EndoMT) limiting scarring, and allowing cardiomyocyte protrusion post cardiac injury.

**Deep evolutionary origin of limb regeneration**

**Igor Schneider** (Louisiana State University)
Sylvain Darnet, Aline Dragalzew, Danielson Amaral, Josane Sousa, Andrew Thompson, Ingo Braasch, Marcus Davis

Regeneration of lost body parts is widespread among animals, but while some animals such as cnidarians and flatworms can regenerate their whole body from just small fragments, others such as nematodes and birds have extremely limited regenerative capacities. The explanation for this extensive variation in regenerative abilities across phyla remains largely unknown. Among land vertebrates, salamanders possess outstanding regenerative capacities and have been widely used as a model for limb regeneration. However, it remains unclear if the mechanisms behind salamander regeneration apply to other vertebrates. Through a comparative analysis of regenerating blastemas, my research team showed deep morphological and molecular similarities between salamander and lungfish regeneration, suggesting that fin/limb regeneration is an ancestral feature of gnathostomes. Furthermore, we used RNA-seq and regeneration assays in various fish species to demonstrate that limb and fin regeneration likely arose early in bony fish evolution and was subsequently lost in amniotes. Our results revealed that the West African bichir (*P. senegalus*) and the axolotl share a regeneration-specific genetic program distinct from development and underscored the importance of an evolutionarily informed approach as powerful strategy for identifying shared genetic and regulatory programs underlying regeneration.

**Retinoic acid breakdown is required for positional identity along the proximodistal axis of the regenerating axolotl limb**

**Timothy Duerr** (Northeastern University)
Melissa Miller, James, Monaghan

Axolotl salamanders possess the capacity for full limb regeneration following amputation at any location along the proximodistal (PD) axis. The positional identity of a regenerating limb is inherent to the blastema, but a molecular mechanism behind how positional identity is established in a regenerating limb has remained elusive. Previous studies have implicated retinoic acid (RA) as a determinant of positional identity along the PD axis, where high levels of RA signaling are found in proximally amputated limbs and low levels of RA signaling are found in distally amputated limbs. It is thought that this difference modulates the expression of mesenchymal cell surface proteins which convey positional identity. Here, we show that a gradient of RA signaling is established via the breakdown of RA by the CYP26 family of proteins, namely CYP26B1. We show that Cyp26b1 is more highly expressed in the mesenchyme of distal blastemas than proximal blastemas. Pharmacological inhibition of CYP26 activity in regenerating limbs with the drug R115866 (talarozole) results in a concentration-dependent proximalization of distal blastemas. We next performed bulk RNAseq on talarozole treated blastemas to uncover transcripts coding for cell surface proteins. We identified Flrt3, Tenm4, and Lphn2, three RA responsive transmembrane proteins that are differentially expressed in the mesenchyme of proximal and distal blastemas. These results point to an RA gradient along the PD axis established by RA breakdown, not de-novo RA synthesis, which leads to expression of transcripts encoding cell surface proteins like Flrt3, Tenm4, and Lphn2 to coordinate PD positional identity in the regenerating limb.

## Information Session: Navigating NIH Peer Review

**Zubaida Saifudeen** (NIH Scientific Review Officer)

This information session will provide an overview on what happens to research grant applications after submission by the Institute (University) and some common grant submission problems. The role of the Center for Scientific Review (CSR) in grant review will be explained, including:

1. Assigning applications to Institutes/Centers and Study Sections

2. How are applications evaluated at a CSR study section meeting
    a. Discussed vs not discussed applications
    b. Summary Statements

# Keynote Lecture

## Regulating tissue regeneration

**Kenneth Poss**
Director of the Duke Regeneration Center, Duke University

Tissue regeneration involves the rewiring of expression of hundreds to thousands of genes, shifting focus of an organ or appendage from function to morphogenesis. Understanding how these changes in gene expression are orchestrated and interpreted is a great challenge in the field of regenerative biology. Dr. Poss will describe findings from his lab that relate to new concepts and applications in gene regulation during regeneration.

**About**
Kenneth Poss uses zebrafish to understand how and why tissue regeneration occurs. As a postdoc, he led the first positional cloning of a gene required for regeneration of amputated fins, and he established zebrafish as a model for innate heart regeneration. With the latter discovery, it became clear that heart regeneration occurs and is efficient in some vertebrates, and that it could be dissected using molecular genetics in a tractable model system. Since then, he and his postdocs, students, and staff have innovated many tools to interrogate tissue regeneration. Dr. Poss reported that heart muscle cells, not stem cells, are activated by injury to divide and directly replace lost cardiac tissue. His lab has a history of research findings on the outer layer of the heart called the epicardium, beginning with discovery of its dynamism upon injury, to its fate-mapping, to its roles in releasing pro-regenerative factors, and to studies describing its own regenerative capacity. His group applied Brainbow-based technology to demonstrate that particularly high proliferative activity by a small number of muscle cells, known as clonal dominance, creates the structure of the adult heart. His lab also identified a key factor important for the process by which zebrafish regenerate spinal cord tissue to reverse a paralyzing injury. Recently, he introduced the concept of tissue regeneration enhancer elements (TREEs), sequences that regulate regeneration programs and can be engineered to enhance tissue regeneration. Dr. Poss was named a Fellow of the American Association for the Advancement of Science in 2019.

**Naproxen exposure during early development inhibits cranial chondrogenesis in axolotl embryos**

**Crystal Rogers** (UC Davis)
Emma Marshall, Tess Leathers

Naproxen (NPX) is a non-steroidal anti-inflammatory drug (NSAID) commonly used to alleviate pain and inflammation via inhibition of the cyclooxygenase (COX1/2) enzymes. Embryonic exposures to NSAIDs are linked to preterm birth, neural tube closure defects, abnormal colonization of enteric nerves, and orofacial malformations. Each of these developmental anomalies may be caused by abnormal neural crest cell development. Neural crest cells are stem-like cells that differentiate into numerous adult tissues including craniofacial cartilage and bone and neurons of the peripheral and enteric nervous systems. We, and others, have identified that Cox1 and Cox2 transcripts are expressed during the early stages of vertebrate embryonic development. Preliminary work in the lab identified that targeted knockdown of COX2 and its receptor, EP3, leads to aberrant neural crest cell maturation in vertebrate embryos. To investigate the phenotypic and molecular implications of NSAID exposure and the development of craniofacial defects, we exposed *Ambystoma mexicanum* (axolotl) embryos to various concentrations of the COX inhibitor, NPX during neural crest cell migration and differentiation stages. We then performed immunohistochemistry (IHC) for markers of neural crest-derived cells. We identified that NPX-exposed embryos have decreased survival, exhibit molecular changes by tailbud stage, and gross anatomic changes by tadpole stages. Specifically, NPX-exposed embryos have reduced expression of SOX9 in neural crest cells, which results in abnormal spatial expression of cells secreting Col2a and absent formation of discrete craniofacial cartilage structures. NPX exposure also appears to disrupt normal RUNX2 expression and patterning in putative precursor cells of the lateral line sensory system. Future work will focus on defining the specific COX signaling pathway effectors that are necessary for normal neural crest development and formation of the craniofacial bone and cartilage.

**Lateral thinking in syndromic congenital disease**

**Christian Mosimann** (University of Colorado School of Medicine, Anschutz Medical Campus)

Syndromic birth defects can present with a wide array of seemingly pleiotropic co-morbidities. Prime examples are rare congenital heart and cardiovascular anomalies that are accompanied by forelimb defects, kidney disorders, and more. Whether multi-organ defects share a developmental link remains a key question with relevance to diagnosis, therapeutic intervention, and long-term care of affected patients. Considering developmental and evolutionary connections between cell fates and organs provides a framework to link congenital disease mechanisms with the observed phenotype spectrum.

The heart, endothelial, and blood lineages develop together from the lateral plate mesoderm (LPM), which additionally also harbors the progenitor cells for limb connective tissue, kidneys, mesothelia, and more. This developmental plasticity with multi-lineage progenitor cells and shared gene-regulatory mechanisms across different LPM lineages has the potential to connect seemingly disparate syndromic defects found in congenital anomalies and to uncover re-activation of developmental gene programs in disease.

We have previously used transgenic reporters in zebrafish and across aquatic chordate models to decode the earliest developmental input that controls LPM gene regulation and of select downstream fates. Harnessing zebrafish transgenes as potent LPM readouts enabled us to test developmental phenotypes of disease-associated genes, to lineage-link cell types affected in congenital heart disease, and to discover re-activated gene regulation signatures active in cancer. Here, I will outline approaches to discover and test gene-regulatory elements as transgenes and will discuss examples of ongoing projects that use novel transgenes to decode cardiovascular disease mechanisms.

**Novel regulators of notochord development suggest a role for purinergic signaling in thoracic spine morphology**

**Brittney Voigt** (The University of Texas at Austin)
Ryan Gray

Opsismodysplasia (OPS; OMIM #258480) is a rare skeletal dysplasia characterized by dwarfism and severe bone malformations. Homozygous or compound heterozygous mutations in inositol phosphatase-like 1 (INPPL1, previously SHIP2) have been implicated in OPS; however, attempts at recapitulating this disorder in mouse models have been unsuccessful. Here, we describe a promising zebrafish model of OPS. Specifically, we identified an allele of the INPPL1 ortholog, inppl1a-stl445, which displays recessive thoracic scoliosis and vertebral bone malformations. The onset of scoliosis in these mutants is preceded by subtle notochord defects including a reduction in the number and volume of vacuolated cells and an overall shortening of the body axis. Embryos treated with AS1938909, a small molecule Inppl1 inhibitor, also displayed a shortened body axis. In addition, we provide preliminary evidence suggesting that a purinergic signaling pathway is involved upstream of Inppl1a function. Specifically, we find that homozygous mutations in the ectonucleoside triphosphate diphosphohydrolase 1 (entpd1-ut31) gene phenocopy the thoracic scoliosis observed in inppl1a-stl445 mutant zebrafish. Furthermore, inppl1a-stl445 and entpd1-ut31 fail to complement in trans, suggesting that these genes function in a common pathway. Given the role of Entpd1 as a nucleotidase protein, we hypothesized that a purinergic receptor may mediate the interaction between extracellular nucleotide catabolism (Entpd1) and intracellular phosphoinositol signaling (Inppl1a). To test this, we performed a CRISPR screen and identified the purinergic receptor P2Y12 (p2ry12) as a potential regulator of thoracic spine development. Altogether, our work in zebrafish highlights a conserved role for INPPL1/Inppl1a in the regulation of bone morphology and spine development. Furthermore, our results suggest that purinergic signaling may play an important role in these processes and may contribute to the etiology of OPS in humans.

**Genetic screening for ethanol-sensitive zebrafish mutants identifies complex signaling interactions in anterior endoderm and jaw development**

**Ben Lovely** (University of Louisville)

Most birth defects are likely due to poorly understood interactions between genetic and environmental factors. To elucidate these interactions, we screened for mutations that enhance ethanol teratogenicity. Ethanol induces similar jaw defects in bmp4 mutants and the forward genetic mutant, au15, neither of which has facial defects when untreated. Ethanol sensitive from 10-18 hpf, the jaw defects in bmp4 mutants are highly variable and resemble those in the anterior endoderm mutant, s1pr2. This suggests a role for Bmp signaling in anterior endoderm morphogenesis. We have previously shown that the endoderm requires Bmp signaling from 10-18 hpf, same as the ethanol-sensitive time window, to regulate an Fgf response in endodermal pouch formation. Surprisingly, while Fgf double mutants display endoderm defects, the jaw is intact and insensitive to ethanol. This suggests that Bmp is regulating additional aspects of endoderm morphogenesis. Here, we show that the Bmp-ethanol interaction disrupts morphology the anterior endoderm with loss of Bmp signaling disrupting signaling centers in the oral ectoderm, oral opening formation and subsequent jaw development. This suggests that ethanol disrupts the complex, Bmp-dependent tissue interactions driving jaw development. The ethanol-induced au15 jaw phenotype is similar to ethanol-treated bmp4 mutants. However, the ethanol-sensitive time window of au15 mutants is from 24-48 hpf, after the ethanol-sensitive time window for bmp4 mutants. Our data show that endoderm morphology is normal but patterning of the neural crest arches is disrupted. We are currently genetically mapping au15 and characterizing the relationship of ethanol-induced jaw loss in au15 and bmp4 mutants. Overall, these data suggest that au15 may function downstream of Bmp signaling as part of an ethanol-sensitive pathway regulating multiple tissue interactions during jaw development. This research is supported by R00AA023560 to BL.

## Key role of autophagy in sustained regeneration and slow aging

Brigitte Galliot (University of Geneva. Switzerland)
Szymon Tomczyk, Nenad Suknovic, Wanda Christa Buzgariu, Chrystelle Perruchoud

Hydra has a long history as a model organism for the study of regeneration and stem cell biology, with three distinct populations of adult stem cells that continually self-renew. Over the past 50 years, Hydra has also been considered a model for the study of aging because *Hydra vulgaris* shows no signs of aging over time while, in contrast, aging can be induced in some strains of *H. oligactis*. Here we propose to discuss recent observations where regeneration and stem cell renewal are irreversibly impaired in aging animals. Indeed, upon induction of gametogenesis, cold-sensitive *H. oligactis* animals (strain Ho_CS) cease to regenerate, lose their active behaviors, and die within three months, an aging phenotype initiated by the loss of interstitial somatic stem cells. This phenotype is not observed in the cold-resistant *H. oligactis* strain (Ho_CR). In aging Ho_CS animals, we found an early and irreversible decrease in epithelial stem cell self-renewal, which remains sustained in non-aging Ho_CR animals. We also identified deficient autophagy in Ho_CS epithelial cells, characterized by an inefficient response to starvation, a deficiency in autophagosome formation, and a poorly inducible autophagy flux. In non-aging H. vulgaris animals, blocking autophagy by reducing ULK1 or WIPI2 expression is sufficient to induce aging. These results highlight the essential role of a dynamic autophagy flux to maintain epithelial stem cell turnover as well as animal regeneration and thus prevent aging.

## Oncogenic allelic interaction in *Xiphophorus* highlights hybrid incompatibility

**Yuan Lu** (Texas State University)

Mixing genomes of different species by hybridization can disrupt species-specific genetic interactions that were adapted and fixed within each species population. Such disruption can predispose the hybrids to abnormalities and disease that decrease the overall fitness of the hybrids and is therefore named as hybrid incompatibility. Interspecies hybridization between southern platyfish (*Xiphophorus maculatus*) and green swordtails (*Xiphophorus hellerii*) leads to lethal melanocyte tumorigenesis. This occurs in hybrids with tumor incidence following progeny ratio that is consistent with two-locus interaction, suggesting melanoma development is a result of negative epistasis. Such observations make *Xiphophorus* one of the only two vertebrate hybrid incompatibility examples in which interacting genes have been identified. One of the two interacting loci has been characterized as a mutant epidermal growth factor receptor. However, the other locus has not been identified despite over five decades of active research. Here we report the localization of the melanoma regulatory locus to a single gene, rab3d, which shows all expected features of the long-sought oncogene interacting locus. Our findings provide insights into the role of *egfr* regulation in regard to cancer etiology. Finally, they provide a molecular explainable example of hybrid incompatibility.

## High mutation levels observed in mitochondrial genomes of damselfish neurofibromatosis tumors

**Elizabeth Schabot** (University of Miami)
Michael Schmale, Wesley Warren

Neurofibromatosis type 1 (NF1) is a common disfiguring genetic disorder affecting 1 in 3000 individuals, with no permanent treatment options. Damselfish neurofibromatosis (DNF) detected in bicolor damselfish, *Stegastes partitus*, shares a striking resemblance to human NF1 tumors. The damselfish virus-like agent (DVLA), which replicates in the mitochondria of the host cell, causes the development of neurofibromas and chromatophoromas that eventually kill the host. DVLA's impact on the mitochondrial genome is not fully understood. To investigate this influence, fourteen RNA sequence libraries including healthy skin, tumored skin, and neurofibromas were processed through a variant detection pipeline. The RNA sequences were aligned to the mitochondrial and the DVLA genomes to determine mutation levels. Additional analysis was conducted on the mitochondrial genome to locate areas of increased mutation that could promote the development of DNF. Neurofibromas and tumored skin demonstrated significantly higher mutation levels than healthy controls in the mitochondrial genome ($p < 0.05$). The highest mutation levels in the mitochondrial genome were observed in complex I and the control region ($p < 0.05$). Substitutions were the dominant mutation type observed compared to indels, and tumor samples demonstrated proportionally more substitutions than normal samples in the mitochondria. The DVLA genome revealed higher mutation levels and proportionally more substitutions than observed in the mitochondrial genome. Mutations in complex I of the mitochondrial genome suggests OXPHOS dysfunction, supporting previous findings. The control region mutations may indicate defects in replication and transcription initiation and machinery, promoting mutation accumulation. Future studies on differential gene and microRNA expression will help elucidate the role of DVLA in mitochondrial dysfunction and how this etiologic agent causes carcinogenesis.

## *Xiphophorus* fishes as models for understanding sex difference in aging

**Peggy Biga** (University of Alabama at Birmingham)
Khalid Freij, Michael Addo, Nicole Riddle

Sex differences in aging are common in many taxa but vary significantly even within species, and the mechanisms that control them are not well understood. Identifying rules that explain sex differences in aging is challenging because phenotypic sex is confounded with many other differences between males and females. This study will determine how genome architecture, organismal biology, and phenotypic plasticity generate sex differences in aging using many taxa, including *Xiphophorus* species. Teleosts, in general, exhibit a diverse range of maximum lifespan, with representatives at both ends of the vertebrate lifespan spectrum: ~0.75 yrs in *Nothobranchius furzeri* and ~205 yrs in *Sebastes auleutianus*. This diversity also extends to sex determination and differentiation, and sexually dimorphic phenotypes. All species of the Poeciliidae are sexually dimorphic in size and morphology, but the magnitude and direct of sexual size dimorphism is variable. Also, Poeciliids represent a variety of sex determination mechanisms, form simple XX-XY or ZZ-ZW systems to polyfactorial sex determination. In this study we have compared the expression of specific target genes, as well as global transcriptomes between old, young, male, and female southern platyfish (*Xiphophorus maculatus*) from two strains exhibiting varying sex chromosomes (Jp163, XX/XY; BpII, WY/YY). This work is building a framework to identify pathways specific to sex and age that will increase our understanding of sex differences in aging phenotypes.

# Workshop on Animal Reference Diets and Nutrition

Rigorous studies and reproducible results are a prerequisite for advances in biomedical research. However, despite the success of aquatic organisms in biomedical research in recent years, standard reference diets, which have the potential to improve scientific rigor and reproducibility, have not been developed or not adopted widely yet. In this workshop, we report about a study across three aquatic research facilities: the Zebrafish International Resource Center (ZIRC) at the University of Oregon, the Kent and Sharpton laboratories at Oregon State University, and the Xiphophorus Genetic Stock Center (XGSC) at Texas State University. We coordinated a 7-month feeding study to compare two commercially available diets with a proposed laboratory reference diet for zebrafish for their roles in general husbandry performance, microbiome development, and fish health in 3 fish species (Medaka, *Xiphophorus*, and zebrafish), and in 3 zebrafish wild-type strains.

We will 1) report our observations with these diets and species, and we will 2) solicit feedback from workshop attendants and 3) discuss what the needs of the aquatic research community are with regards to the development of reference standard diets for the aquatic species represented at this AQMHD conference.

**Introduction to mutrition and standard reference diet**

**Steve Watts** (University of Alabama at Birmingham)

**Study overview and reference standard diet testing at the ZIRC**

**Zoltan M. Varga** (Zebrafish International Resource Center, University of Oregon)

To develop and optimize open-formula reference diets, an iterative process is needed, where the reference diets are repeatedly tested and improved based on the husbandry and research requirements established by the research communities. As a first step in this iterative process, we tested a currently available open-formula, high-fat diet for rearing of larvae and juvenile, and a low-fat diet for maintaining and breeding adults. These diets were generously provided by Steve Watts and his colleagues at the University of Alabama at Birmingham, who have developed and published candidate reference diets for zebrafish. Specifically, we assessed growth, fertility, and susceptibility to prevalent zebrafish diseases in three zebrafish lines, AB, TU, and Cooch Behar (CB) between 1-month and 7-months post fertilization. The AB stock for this study was generated *Pseudoloma neurophilia* free (SPF) and split at 1 month of age for parallel testing at ZIRC and the Sharpton and Kent laboratories at Oregon State University. We observed physical characteristics of the reference diet that can be improved to maintain it suspended longer in the water column for better feeding access. However, our results at ZIRC indicate that there are no significant differences in the husbandry and health outcomes we analyzed, indicating that the proposed Watts Reference Diet performs at least as well or better as the two commercially available diets we evaluated in parallel.

**Assessment of various standard fish diets on growth, fecundity, and microbiome of *Xiphophorus maculatus* (platyfish) and *Oryzias latipes* (Medaka)**

**Yuan Lu** (Xiphophorus Genetic Stock Center, Texas State University)
Crystal Russo, Erika Soria, Merritt Drewery, Carolyn Chang, Camila Carlos-Shanley, Markita Savage, Will Boswell, Ronald Walter, Lindsey Sanchez, Zoltan Varga, Michael L. Kent

Several small freshwater fish species are utilized as models for human conditions and disease in biomedical research. Research animal diets are generally tailored to optimize growth, fecundity, and produce healthy research animals. However, a lack of reference diets presents a barrier in comparative studies between aquatic animal models and even among laboratories using the same species. Therefore, the objective of this study was to determine feeding regime and dietary effects on growth and fecundity in two commonly used freshwater fish, platyfish and medaka. From one through six months of age, platyfish and medaka were fed one of three feeding regime/diets: 1. A custom feeding regime consists of commercial flake food, beef liver paste, and live brine shrimp (CON); 2. a commercially available zebrafish diet, Gemma (GEM); and 3. a laboratory defined reference feeding regime (WAT). Weight, size, brood numbers, and survival rates for both species were measured, and gut microbiome of platyfish were assessed using 16S rRNA sequencing monthly. Numbers of platyfish fry and hatch rate of medaka embryos were also determined. We observed that custom feeding regime (CON) fed platyfish and medaka grew larger, exhibited a higher survival rate, and had higher fecundity than WAT or GEM fed fish. In addition, gut microbiome of platyfish subjected to the two zebrafish reference exhibit major differences from that of CON diet. These observations suggest diets and regimes designed for zebrafish are not optimal to maintain platyfish or medaka. Thus, base diets, with clearly defined components and regimes, need to be developed with compositions that can be adjusted in a species-specific manner.

**Impact of diet on growth and disease susceptibility in zebrafish**

**Michael Kent** (Oregon State University)
Michael J. Sieler Jr, Kristin Kasschau, Thomas J. Sharpton

There is now an increasing popularity in microbiota-targeted studies and research on infectious diseases using the zebrafish model. There is a lack of consensus regarding zebrafish husbandry practices involving type of diet. This is a concern because diet influences gut microbiota composition, and may influence physiological variation and susceptibility to infectious diseases. It is not known whether standard zebrafish diets manifest in distinctive gut microbiota composition as well as fitness, nor is it known how diet influences pathogen burdens in zebrafish. Pertaining to this study, we are particularly interested in Mycobacterium chelonae because infections are very common in zebrafish and it is transmitted by the oral route of exposure. We conducted experiments with three zebrafish laboratory diets; two commercial diets Gemma and Ziegler + Spirulina) and a defined diet (Watts). Fish fed the Zielger diet showed significantly greater weight and condition factor, driven by large females. Some fish were experimentally exposed to M. chelonae by IP injection, and the fish from the Zielger group showed a higher prevalence of infection compared to the other two groups. Microbiome analysis showed that Watts diet fed fish had the lowest alpha diversity scores, and body condition score was negatively associated with alpha diversity score in the Ziegler group. Watts diet fed fish had the highest beta diversity scores and Gemma diet fed fish had the lowest. The microbiome composition of Ziegler diet fed fish uniquely stratified by body condition score at 6 mo post-feeding. Additionally, 421 bacterial taxa were differentially abundant across diets. Our results demonstrate that variation in husbandry practices relating to diet impacts the successional development of the zebrafish gut microbiome. Our study also indicates that the role of diet in zebrafish research, particularly with disease and microbiome studies, should be considered.

**The impact of diet on the zebrafish gut microbiome**

**Tom Sharpton** (Oregon State University)

Despite the long-established importance of zebrafish as a model organism and their increasing use in microbiome-targeted studies, relatively little is known about how husbandry practices involving diet impact the zebrafish gut microbiome. Given, the microbiome's important role in mediating host physiology and the potential for diet to drive variation in microbiome composition, we sought to clarify how three different dietary formulations that are commonly used in zebrafish facilities impacts the gut microbiome. We reared 60 fish on each diet throughout their lifespan and compared the composition of their microbiomes at both 3- and 6-months post fertilization. Our analysis finds that diet has a substantial impact on the composition of the gut microbiome at both 3- and 6-months of age. Moreover, the developmental dynamics of the microbiome differ as a function of diet. We further evaluated whether the 6-month post fertilization microbiome compositions that result from dietary variation are differentially sensitive to infection by a common laboratory pathogen: Mycobacterium chelonae. Our analysis finds that the impact of M. chelonae infection on the gut microbiome differs as a function of diet, especially for moderate and low abundance taxa. Overall, our results indicate that diet drives the successional development of the gut microbiome as well as its sensitive to exogenous exposure. Consequently, investigators should carefully consider the role of diet in their microbiome zebrafish investigations, especially when integrate results across studies that vary by diet.

**Convergence, divergence, and cooption in the adaptive immune system in the "other" major vertebrate lineage**

**Chris Amemiya** (University of California, Merced)

I will describe our work in a biological system where there has been substantial convergence as well as divergence and cooption: the adaptive immune system. Specifically, I will describe the antibody system in the lamprey, a representative of the agnathan lineage. Extant agnathans include the lampreys and hagfishes and are referred to as "jawless vertebrates". The split between jawed vertebrates and jawless vertebrates occurred around roughly 550 million years ago and was coincident with the emergence of two vastly different means for defense to pathogens. These two systems are comprised of strikingly different genetic and molecular toolkits for responding to pathogens using cellular and humoral responses that are based on antibodies. However, the lamprey's immune system does not utilize the immunoglobulin-based system found in the jawed vertebrates, but rather utilizes receptors based on leucine rich repeat modules, the variable lymphocyte receptors (VLRs). There are clear parallels between the two systems despite the vastly different genetic and molecular toolkits they respectively possess. I will discuss how the VLRs are paradoxically also used in early embryonic development, how the "rearranging" mechanisms may have emerged evolutionarily, and how we can coopt and exploit the system for use in synthetic biology and the generation of monoclonal antibodies.

**An Enteroendocrine-vagal sensory pathway that transmit gut bacterial signal to the brain**

**Lihua Ye** (The Ohio State University)
Melissa Brewer, Rodger Liddle, John Rawls

The intestine harbors complex and dynamic microbial communities that contribute significantly to host health. However, mechanisms by which the intestine perceives distinct microbial species and relays that information to the brain remain unresolved. Enteroendocrine cells (EECs) are specialized sensory epithelial cells in the intestine that detect nutrients and other chemicals in the intestinal lumen. Recent studies in mice show EECs synapse directly with vagal neurons and that nutritional stimuli in the intestine can activate this neuroepithelial circuit. Despite these central roles of EECs in intestinal nutrient sensing, it remained unknown if EECs sense bacteria and what the downstream effects may be. To test the impact of bacteria on EECs in vivo, we developed novel zebrafish genetic models and established high-resolution calcium imaging approaches to record spatial-temporal EEC activity in the whole animal and track EEC function in real-time in vivo. We screened a panel of bacteria and identified the bacterium *Edwardsiella tarda* (*E. tarda*) significantly elite EEC activity through activating the transient receptor potential ankyrin 1 (Trpa1). Using the optogenetic and in vivo vagal calcium imaging, we have discovered that intraluminal *E. tarda* bacteria directly activate vagal sensory neurons to modulate brain activity through EECs. Zebrafish whole-brain activity analysis revealed that activation of Trpa1+EECs alters neuronal activities that are involved in emotion response. Using the single-cell RNA sequencing, we revealed that the Trpa1+EECs specifically enriched in a neuronal peptide that is involved in pathogen defense. Blocking this neuronal peptide signaling inhibits Trpa1+EECs transmit bacterial signals to vagal sensory neurons. Collectively, using new approaches in zebrafish model, our data establish a new molecular pathway by which EECs regulate brain activity through vagal sensory neurons in response to specific microbial signals.

**Using zebrafish to disentangle the impact of environmental exposure on host-microbiome interactions**

**Thomas Sharpton** (Oregon State University)

Extensive evidence demonstrates that the gut microbiome contributes to vertebrate physiology and that gut microbes can mediate how environmental factors, including nutrients, pollutants, and parasites, impact health. Consequently, the effort to manage the health of vertebrates benefits from understanding how different environmental factors interact with the microbiome to affect physiology. This effort, however, remains bottlenecked by the extensive scope and variation of environmental factors to which vertebrates are exposed. Researchers increasingly turn to the zebrafish model system to study these interactions, as it affords access to features that alleviate this bottleneck, including large sample sizes, automated exposure and phenotyping platforms, and short generation times. In this presentation, I summarize the utility and potential for the zebrafish model system to accelerate our understanding of how exposures interact with the microbiome to impact health. I also introduce key zebrafish research analytical and experimental tools we and others innovated to this end, including longitudinal sampling designs, high-throughput germ-free fish assays, and strategies for multi-omic data integration. Finally, I will discuss recent research we have conducted that exemplifies the utility and impact of this model system for microbiome research. This discussion will include our recent characterization of how exposure to polycyclic aromatic hydrocarbons affects zebrafish gut microbiome assembly to impact larval fish behavior, as well as our use of longitudinal multi-omic sampling to clarify mechanisms through which gut microbes may mediate helminthic infection and gut inflammation outcomes in adult zebrafish.

**Social-like behavior is still inducible in the evolutionarily asocial cavefish by a dietary intervention**

**Masato Yoshizawa** (University of Hawaii at Manoa)
Crystal Valdez, Jaimee Kato, Emma Doy, Amity Tran, Jia Cashon, Kate Coyle, Aldo Carmona Baez, Reade Roberts, Motoko Iwashita

In many animal phyla, the gut microbiota is known to regulate brain outputs, including social behaviors. However, the precise molecular mechanism of how microbes' metabolites interact host's genetic variation and modify brain function is largely unknown. To implement host genetic variation, we strategically chose the Mexican tetra, *Astyanax mexicanus*, consisting of cave-dwelling (cavefish) and the riverine morphs (surface fish). Cavefish behavioral phenotypes and genetics show many parallels with patients with autism, including asociality and restricted repetitive behavior. In contrast, surface fish show typical behaviors. Cavefish genes also show many up or down regulations in the same directions seen in the brains of patients with autism (>58.5% of 3152 orthologs), compared with surface fish. In contrast, other proxy systems (BTBR mouse, shank3 knock-out mouse, and iPS cell-derived neural cells) have shown much less overlap (<11%). Also, the gut of cavefish showed significant depletion of lipid-processing Firmicutes, similar to patients with autism. To reveal the link among the host genes, gut microbiota, and brain functions, we shifted the gut microbiota with antibiotics or dietary interventions. After the one-month oral treatment, the antibiotics slightly modified sleeplessness but did not induce detectable changes in other behavioral traits in either surface or cavefish. In contrast, cavefish significantly increased social-like behavior under the ketosis-inducing ketogenic diet, while the ketogenic diet changed gut microbiota composition. We currently focus on the major metabolites of ketosis, butyrate, and are addressing its effect on behavior, gut microbiota, and brain transcriptome, by comparing two genetically diverged surface and cave populations.

**The role of altered biotransformation pathways in the rapid adaptation of Gulf killifish to legacy pollutants**

**Ramon Lavado** (Baylor University)
Marco E. Franco, Cole W. Matson, Karla M. Johanning

Chronic exposure to pollution may lead populations to display evolutionary adaptations associated with cellular and physiological mechanisms of defense against xenobiotics. An example of this scenario corresponds to Gulf killifish (*Fundulus grandis*) populations inhabiting the Houston Ship Channel (HSC), Texas, USA, which have been documented to be pollution-adapted. In the present study, we explored two F. grandis populations with different exposure and evolutionary histories for their ability to biotransform polycyclic aromatic hydrocarbons (PAHs) and conducted a comparative evaluation of in vitro and in vivo approaches to describe differences in: 1) biotransformation enzyme activity, 2) intrinsic hepatic clearance, and 3) production of metabolites. Pollution-adapted *F. grandis* presented significantly lower CYP1A activity but higher CYP2C9-like activity than non-adapted fish. Similarly, in pollution-adapted fish, in vitro hepatic clearance rates were significantly lower, particularly for females and high-molecular weight PAHs (chrysene, benzo[k]fluoranthene, and benzo[a]pyrene). Subsequent experimentation involving intraperitoneal injections of benzo[a]pyrene and bile analysis showed the presence of 1 hydroxy-benzo[a]pyrene but with no significant population differences. Contrarily, 9 hydroxy-benzo[a]pyrene and benzo[a]pyrene-4,5-dihydrodiol presented higher concentrations in the bile of pollution-adapted fish relative to non-adapted individuals. These results provide significant evidence of population- and sex-specific biotransformation differences in F. grandis and demonstrate the importance of exposure and evolutionary histories in shaping organisms' responses to pollution.

**Déjà vu: exploring 'replicate' radiations of perciform fishes to understand the genetic and developmental origins of key traits and adaptation to extreme environments**

**Jacob Daane** (University of Houston)
H. William Detrich III, Matthew, Harris, Andres Aguilar, Michael Sandel

Central to adaptative radiation is the origin of traits that facilitate access to novel niches, yet the genetic and developmental mechanisms underlying the appearance of these traits are poorly understood. Over the last 100 million years, perciform fishes have undergone multiple independent adaptive radiations in response to similar ecological and environmental opportunities. Can such ‘replicate’ radiations help us to disentangle lineage-specific signals from more universal mechanisms of trait evolution? Here, we discuss comparative genomics in two perciform radiations of the marine Antarctic notothenioids and the freshwater sculpins of Lake Baikal. For both radiations, the main axis of ecological diversification has been the water column, from shallow benthic ancestors into deep-water and pelagic lineages. The pelagic lineages of both radiations have evolved despite the absence of a swim bladder, the buoyancy organ of most fishes. Instead, both clades have evolved improved buoyancy through reduction in skeletal density and accumulation of corporeal lipids. Using whole genome and targeted sequencing approaches, we have assembled genomic data from 44 notothenioid species, 24 Baikal sculpins and several outgroup lineages, which enable us to track macroevolutionary trends and to discover patterns of protein coding and gene regulatory evolution. We will compare the two radiations and describe genetic signatures underlying buoyancy adaptations and their implications for understanding human diseases. Supported by US NSF 1955368 (JMD, HWD), 2001584 (MPH) and 1557147 (AA and MS).

**Web-based reference atlas of *Daphnia magna***

**Khai Ang** (Penn State College of Medicine)
Mee Ngu, Daniel Vanselow, Carolyn Zaino, Maksim Yakovlev, Alex Lin, Dilworth Parkinson, Margaret Beaton, John Colbourne, Keith Cheng

We are creating web-based reference atlas for whole aquatic meso-organisms (0.5 to 10mm in size) to anchor comprehensive anatomical and microanatomic phenotypic data across organ systems at various developmental stages, to serve as a foundation for automated phenotyping and spatial biology. Our goal is to use reference atlases for unbiased assessment of physiological and pathological changes across all cell and tissue types in genetically modified or environmentally exposed organisms. Daphnia is a commonly used model organism for toxicity testing and environmental monitoring. We present a *Daphnia magna* atlas containing histology and histotomography, a soft tissue micro-tomography (microCT) that is a 3D form of histology. The atlas (http://daphnia.io/anatomy) contains virtual slides of 5-micron thick slices at up to 40X magnification (~0.25-micron/pixel) presents the anatomy with anatomical structures labelled in all three orthogonal planes. High-resolution microCT images can be represented as 3D renderings and digital histology-like sections (0.5-micron pixels) in any plane, angle, and slice thickness from the reconstructed image stacks. The thickness of maximum intensity projections (MIPS) can be adjusted (0.5 to 100s of microns) to allow the visualization of anatomical structures of different thicknesses, while maintaining resolutions that are comparable to histology. Structure segmentation is being done both manually and using supervised machine learning, followed by qualitative and quantitative analysis. We are extending our Daphnia atlas digital workflow, first, to the zebrafish atlas (http://bio-atlas.psu.edu) and then to other organisms. New combinations of achievable sub-micron voxel resolution and large field-of-view based on newly created microCT imaging instrumentation will make the reference atlas a versatile tool for comprehensive and quantitative histological analysis of meso-scale organisms in ecotoxicity studies and environmental monitoring.

**Transgenerational benzo[a]pyrene exposure as a risk factor for reduced bone mineralization**

**Frauke Seemann** (Texas A&M University-Corpus Christi)
Jiezhang Mo, Miles Teng Wan, Richard Kong, Doris Au

Fragility fractures due to bone loss in adults are projected to exceed $25.3 billion in U.S. medical costs by 2025. Reports in the literature and our preliminary data indicate contributions of parental polycyclic aromatic hydrocarbons exposure to reduced bone health and increased fracture risk in the offspring. Our lab has demonstrated that parental exposure to benzo[a]pyrene (BaP) at environmentally relevant doses impairs bone formation in the offspring of exposed Japanese medaka (*Oryzias latipes*), a widely utilized and tractable ecotoxicology fish model. It is hypothesized that epigenetic mechanisms are responsible for the bone phenotype inheritance. In a multi-biological level approach, vertebra compression (development) and reduced bone thickness (adult male) at the tissue level were likely associated with reduced osteoblast differentiation and activity, which was revealed at the cellular level through temporal and spatial assessment of bone cells in transgenic medaka strains. Analysis of the bone tissue transcriptome revealed the deregulation of (i) bone metabolism canonical pathways and (ii) BaP-responsive signaling pathways indicating the disruption of the osteoblast-osteoclast interplay during bone metabolism and associated miRNAs on the molecular level. Modified histone- and DNA methylation pattern were identified in bone tissue and bone genes during development and in adult organisms indicating an ancestrally BaP-induced modification of the epigenetic profile in the offspring. The sperm methylome analysis indicated a reduced contribution of paternal DNA methylation to the inherited bone phenotype. The presented data will shed light on the genetic and epigenetic pathways and provide a scientific basis to reassess the impact of environmental BaP on public and environmental health, foreshadowing strategies for early detection of ancestral exposure and reduced bone mineralization.

# Cryopreservation and Open Hardware Workshop

Development of efficient and reliable germplasm repositories is critical for preservation of genetic resources of aquatic model organisms that are vital to advancing biomedical research. Community participation in establishment of sperm repositories is the most efficient strategy to ensure preservation and distribution of valuable lines. However, the most significant barrier in repository development is the lack of cryopreservation capability and reproducibility across the research community, posing great risks of losing advances developed from billions of research dollars.

The emergence of open scientific hardware has fueled a new movement across biomedical research communities. To facilitate this movement, the National Institutes of Health has launched a 3D Print Exchange platform (https://3dprint.nih.gov), including >12,000 3-D models to be shared among users. With the increasing accessibility of consumer-level 3-D printers, open hardware devices for cryopreservation can be designed, and the files distributed to community members who can use them reliably freeze and evaluate samples in a standardized and reproducible way.

To assist utilization of these new technologies, the Aquatic Germplasm and Genetic Resources Center (www.aggrc.com) is hosting a workshop at the 2022 Aquatic Models for Human Disease meeting. The workshop will include an introduction to 3-D printing applications in germplasm cryopreservation and repository development, and provide user interaction with various cryopreservation devices, 3-D printing process, and outreach kits. Attendees will have the opportunity to receive 3-D printed souvenirs.

**Germplasm repositories and community development for genetic resources of aquatic species: A role for open technologies**

**Terrence Tiersch** (Louisiana State University)

Globally, there are immense challenges that defy resolution despite offering tremendous opportunities. Development of germplasm repositories to protect genetic resources of aquatic species is such a challenge. Despite 70 years of cryopreservation research, fish and shellfish have only minimal frozen collections although there are thousands of publications, primarily addressing creation of freezing protocols. This is in stark contrast to livestock such as dairy for which massive collections drive multi-billion dollar global markets for improved genetics. The lack of repositories suppresses advances across aquaculture, conservation programs, natural fisheries, biomedical models, and efforts to address food security and poverty alleviation. Recognition of this as an immense challenge (not addressed by current approaches) is a step towards resolving it. Because problems such as this are beyond the resources of single entities, other models are required to address them. An emerging model involves use of distributed networks to combine the efforts of large, interconnected communities that share common motivation. This approach was used to develop the Linux operating system in the 1990s through open-source software development driven by thousands of volunteer computer programmers. This sharing and community-based approach was in response to the limitations of proprietary development. The success of Linux provided impetus for other open-source projects, and the experience gained opened doors to expand distributed development. This spirit has emerged in renewed form with new consumer-level design and fabrication technologies that can enable study, distribution, production, modification, and improvement (based on licensing agreements) of computer-aided design files shared over the internet. As such, these technologies provide a powerful alternative to traditional research and proprietary development to combine efforts across multiple communities to establish repositories.

**Open Hardware Demonstrations**

**Jack Koch, Lucia Arregui, Maria Teresa Gutierrez-Wing** (Louisiana State University)

1) Equilibrium cooling devices

2) Vitrification and quality management devices

3) 3-D Printing, outreach, and community interaction

**An open-hardware movement to support development of germplasm repositories for aquatic biomedical models: Conclusion and quiz with awards**

**Yue Liu** (Louisiana State University)

Development of efficient and reliable germplasm repositories is critical for preservation of genetic resources of aquatic model organisms that are vital to advancing biomedical research. However, the most significant problem in repository development is the lack of reproducibility, posing great risks of losing valuable lines developed from billions of dollars research investment. Open hardware allows users to gain access to technologies through open-sharing mechanisms and enable individual contributions for improvement to facilitate community-scale standardization that would rarely be achieved through proprietary technologies. Our work recognizes 14 categories of open hardware for a cryopreservation processing pathway, and 6 categories for a corresponding quality management pathway to address the two impediments to establishing repositories among resource centers and research communities. Although cryopreservation protocols have been established through basic biological research, low-quality samples are often produced that cannot be revived because of a lack of affordable, standardized, and reliable hardware to process samples along a production pathway. In addition, there are currently no cost-effective hardware options to enable quality management, including quality assessment for accurate evaluation, quality assurance mechanisms for prevention of defects, and quality control for elimination of inferior materials. Although some of these issues can be alleviated by commercial solutions, most laboratories are not willing to purchase expensive equipment (that can cost tens of thousands of dollars) when germplasm banking is not a focus or obligation of their work. Through open hardware, individuals can fabricate standardized devices in-house with low cost, offering opportunities to begin or improve germplasm preservation, and facilitate repository development with community efforts through aggregated high throughput.

# Keynote Lecture

## Sex-dependent, epigenetic, and multigenerational adverse outcomes in zebrafish following parental benzo[a]pyrene exposure

**Kristine L. Willett**
Chair of BioMolecular Sciences, University of Mississippi

Benzo[a]pyrene (BaP), a polycyclic aromatic hydrocarbon (PAH), represents a class of ubiquitous environmental contaminants derived from the incomplete combustion. Human exposures to PAHs are associated with developmental, reproductive and multigenerational deficits, but the molecular mechanisms have not been fully elucidated. We used zebrafish to study BaP's sex-dependent and multigenerational developmental toxicities assessed at different life-stages and variable routes of parental exposure (water vs diet). Following a continuous waterborne BaP parental and larval exposure, BaP significantly reduced egg production, F1 survival and global DNA methylation. Functional ontology analysis of differentially expressed genes (DEG; RNASeq) revealed that many disease pathways were consistent with observed phenotypes, including organismal death, growth failure, abnormal morphology of embryonic tissue, congenital heart disease, and disrupted neuritogenesis. Subsequent experiments using a dietary parental exposure resulted in dose-dependent multigenerational adverse phenotypic impacts in F1 and F2 offspring with body deformities being the most severe. Craniofacial structures were not significantly affected in F1 fish but emerged as significant deformities in F2 fish. A crossover breeding design following a parental dietary BaP exposure yielded F1 cohorts of control and three crosses from BaP-exposed parents. Significant hyperactivity (light-dark assay) was detected in F1 96 hpf fish resulting from both crosses with paternal BaP exposure. Gene expression and DNA methylation (RRBS) was measured in F0 gonads and 10 hpf F1 embryos resulting from each cross. F1 embryos from the BaP male x control female cross had the most differentially methylated regions and DEGs. Overall, we found that BaP exposure results in adverse phenotypic outcomes that are multigenerational, and paternal exposure contributes most significantly to these adverse outcomes. Supported by NIEHS 1R21ES030154.

**About**

Dr. Willett's research has been funded over the years by NIDA, NIEHS, NOAA and the Army Corps of Engineers. Her lab studies the developmental, reproductive and multigenerational impacts of cannabinoids and benzo[a]pyrene exposure using fish models. She also has studied nanosilver mechanisms of toxicity and the consequences of the Deep Water Horizon Oil Spill on oysters. She is also part of a multidisciplinary team of researchers helping to engage Mississippi communities to decrease lead exposure via drinking water. Throughout her career she has led research projects which were designed to fundamentally understand the molecular mechanisms underlying toxicity and/or shed light on the potential adverse outcomes due to relevant anthropogenic contamination.

Dr. Willett is active in both the Society of Environmental Toxicology and Chemistry (SETAC) and the Society of Toxicology (SOT) where she is especially involved in aspects of toxicology undergraduate education.

**The nudibranch *Berghia stephanieae* is a new experimental system for studying molluscan brain development**

**Deirdre Lyons** (UC San Diego)
Park Masterson, Hereroa Johnston, Neville Taraporevala, Jessica Goodheart, Carl Whitesel, Grant Batzel, Vanessa Barone, Rose Fiorenza, Antonia Bock

Nudibranchs are shell-less, uncoiled, sea slugs that are well known for many unique biological and behavioral attributes. A handful of species have been used extensively as experimental systems for adult neuroethological studies for many years. They were particularly well-suited for such investigations because of the large, identifiable neurons in their brains, and their distinct swimming behaviors. However, little is known about early life stages. For example, how the nervous system controls behavior during embryonic, larval, and juvenile stages remains unclear, because it is not easy to work with the embryos of commonly used species. Understanding the link between neuronal development and animal behavior at early life stages is essential for comparisons with juvenile and adult behaviors. Some key questions about brain function and development remain technically challenging to answer because: 1) the species currently used for neuroscience are not easily cultured in the laboratory, and 2) functional genomics tools like transgenesis, sequenced genomes, or gene knock out methods are not available. To address these deficiencies, we chose the aeolid nudibranch *Berghia stephanieae* as a new experimental research organism. We established methods for culturing animals egg to egg on the bench top, defined a detailed staging system from zygote to reproductive adults, showed that the embryos can be microinjected with nucleic acids, and produced a chromosome-level genome assembly. With access to developmental material, we addressed several long-standing questions about nudibranch nervous system development, including how the brain grows and consolidates during metamorphosis, and at what stage identified neurons can first be detected. This body of work sets the stage for developing modern tools, such as genetically encoded biosensors, to study neural circuits at all stages of the lifecycle.

**Regulation of neuron and muscle sub-type traits in the non-vertebrate marine chordate *Ciona***

**Alberto Stolfi** (Georgia Institute of Technology)

Mutations in the human EBF3 gene, encoding a transcription factor in the Collier/OLF1/Early B Cell Factor (COE) family, have been implicated in a growing number of previously unexplained congenital neurodevelopmental disorders. Our work in *Ciona robusta* and other tunicates has revealed different roles for EBF in the regulation of several processes in neural and muscle development in the sister group to vertebrates. Here we present some of our recent findings on the myriad functions of EBF in *Ciona* development, ranging from neurotransmitter selection, neuromuscular synapse maturation, and myoblast fusion. We also present a curious case of reduced EBF expression in the developing nervous system of a tunicate species that has evolved tailless, non-swimming larvae that bypass the dispersal phase present in the typical tunicate life cycle.

**Whole brain imaging in the blind cavefish reveals anatomical and functional evolution of a precise neural circuit modulating light responsiveness**

**Erik Duboue** (Florida Atlantic University)

Evolution of the brain is a fundamental principle to adaptation. Comparative studies have enhanced our understanding of evolution impacts the vertebrate brain, yet large divergence times and lack of molecular and genetic tools have impeded our understanding of how changes to anatomy and function of precise neuronal circuits causes adaptive behaviors to emerge. We have isolated a neural circuit underlying light sensitivity in the blind Mexican cavefish, *Astyanax mexicanus*, and are using this as a model to study how evolution alters behavior. We find that both cave and surface fish have retained ability to detect change in photo-illumination, yet whereas surface fish become hyperactive when lights are removed, cavefish because hyperactive when lights turn on, presumably an adaption to evolution in the perpetual darkness of a cave. Using and novel whole brain neuroanatomical atlas, we have isolated a neural circuit modulating light responsiveness. We assessed the volumes of every known neuroanatomical locus of the brain. Further, using functional mapping of neuronal activity, we have isolated a region of the telencephalon whose neuronal activity may explain the difference in light preference. To determine the spatiotemporal dynamics of neuronal activity in this region we are using stable GCaMP *Astyanax* and performing functional 2 photon imaging. These findings reveal how changes to a defined neuronal circuit modulates light sensitivity and establishes a model where the genetic changes in natural populations can be associated with changes in both anatomy and function of the vertebrate brain, and lead to an evolutionary behavior adaptation.

***Aplysia californica* as a model for viral infection in neurological aging**

**Nicholas Kron** (University of Miami)
Lynne Fieber, Michael Schmale

*Aplysia californica* is a well-studied neural model, particularly useful in the study of individual neurons and simple neural circuits. Recently, we described signatures of inflammation, endoplasmic reticulum (ER) stress, and possibly immune activation in the sensory neurons of aging Aplysia. While these signatures are known to associated with normal aging, they are also consequences of viral infection. Recently, a nidovirus, dubbed Aplysia Abyssovirus (AAbV), was identified in publicly available Aplysia RNA sequencing datasets from a variety of tissues, including the nervous system. Reevaluation of datasets from our previous studies revealed viral load increases in Aplysia sensory neurons with age. Furthermore, analysis suggests viral load better describes age-associate transcriptional change than does chronological age. Enriched processes include those typically dysregulated by other nidoviruses, e.g., coronaviruses. Slow neurological infection has been hypothesized to be a contributor of neurological aging in humans, and viruses are routinely associated with neurodegenerative disease such as Alzheimer's disease. These early results suggest the utility of Aplysia sensory neurons as a novel model of viral-associated neurological aging.

**Common threads of spine morphogenesis in zebrafish**

**Ryan Gray** (University of Texas at Austin)

Scoliosis is defined as one or more atypical curvatures of the spine along the coronal or sagittal plane and is the most common form of structural spinal deformity affecting children worldwide. Using forward and reverse genetic approaches we have identified several mutant loci that cause a variety of scoliosis subtypes in zebrafish. Our analysis of several mutants displaying whole-body scoliosis, characterized by atypical curvatures in both dorsal-ventral and medial-lateral axes has helped to define a connection between motile cilia physiology and the assembly of the Reissner fiber. The Reissner fiber is an extracellular thread of proteinaceous material bathing within the cerebrospinal fluid of the spinal cord central canal, which we demonstrated has dynamic treadmilling properties as it is continuously secreted from the brain and resorbed at the tip of the tail in zebrafish.
We show that mutations in the putative motile cilia component genes, kif6 and dnah10, caused disruptions of motile cilia physiology, disassembly of the Reissner fiber, and whole-body scoliosis. Moreover, we show that mutations that disrupt putative disulfide bonds of Scospondin - the major protein component of the Reissner fiber - also led to the disassembly of the Reissner fiber and the onset of whole-body scoliosis. This suggests a model where cilia motility, possibly via generation of laminar fluid flow, is necessary for the normal dynamics and continuous assembly of the Reissner fiber. Interestingly, mutations in both KIF6 and DNAH10 have been associated with pleiotropic disorders in humans including intellectual disability, hypotonia, and in some cases, scoliosis. Altogether this work suggests that our analysis of whole-body scoliosis in zebrafish may contribute to our understanding of the pathogenesis of scoliosis phenotypes, which are a common sequela of neuromuscular disorders in humans.

**Skeletal repair and regeneration in ageing zebrafish**

**Chrissy Hammond** (University of Bristol, UK)
Lucy McGowan, Qiao Tong, Erika Kague

Zebrafish have an incredible regenerative capacity and are capable of perfectly reforming many tissues repeatedly. In many organs and tissue there are strong parallels between the mechanisms of tissue repair, regeneration and early embryonic development. Like humans, zebrafish show increased susceptibility to skeletal pathologies as they age; including osteoporotic fractures, osteoarthritis of their joints and intervertebral disc degeneration. Using fin and scale fracture and regeneration assays we show that regenerative capacity and repair are reduced in ageing fish. By manipulating the innate immune system we show that macrophages play a role in skeletal regeneration and that neutrophils, unexpectedly, play a beneficial role during fracture repair stabilizing the fracture in the earliest stages of repair.

**Urotensin-II-related peptides, Urp1 and Urp2, control zebrafish spine morphology**

**Elizabeth Bearce** (University of Oregon)

The spine provides structure and support to the body, yet how it develops its characteristic morphology as the organism grows is little understood. This is underscored by the commonality of conditions in which the spine curves abnormally such as scoliosis, kyphosis and lordosis. Understanding the origin of such spinal curves has been challenging in part due to the lack of appropriate animal models. Recently, zebrafish have emerged as promising tools with which to understand the origin of spinal curves. Using zebrafish, we demonstrate that the Urotensin II-related peptides (URPs), Urp1 and Urp2, are essential for maintaining spine morphology. Urp1 and Urp2 are 10-amino acid cyclic peptides expressed by neurons lining the central canal of the spinal cord. Upon combined genetic loss of Urp1 and Urp2, adolescent-onset planar curves manifested in the caudal region of the spine, akin to a lordosis-like condition. Highly similar curves were caused by mutation of Uts2r3, an URP receptor. Quantitative comparisons revealed that Urotensin-associated curves were distinct from other zebrafish spinal curve mutants that more closely reflected idiopathic scoliosis or kyphosis. Last, we found that the Reissner fiber, a proteinaceous thread that sits in the central canal and has been implicated in the control of spine morphology, breaks down prior to curve formation in an idiopathic scoliosis model but was unperturbed by loss of Uts2r3. This suggests a Reissner fiber-independent mechanism of curvature in Urotensin-deficient mutants. Overall, our results show that Urp1 and Urp2 control zebrafish spine morphology and establish new animal models of lordosis-like curves.

**From tank to bedside: Transcriptome profiling in a medaka osteoporosis model identifies a novel blood marker that reliably predicts risk for osteoporotic hip fractures in men**

**Christoph Winkler** (National University of Singapore)
Quang Tien Phan, Wen Hui Tan, Kevin Chua, Aizhen Jin, Woon-Puay Koh

Healthy bone requires reciprocal communication between bone-forming osteoblasts and bone resorbing osteoclasts to maintain bone homeostasis. Any communication failure results in bone diseases, such as osteoporosis, where uncontrolled osteoclast activity results in excessive bone resorption and bone fractures. To identify novel signalling factors implicated in osteoblast-osteoclast communication, our lab had earlier conducted transcriptome profiling of bone cells in a medaka osteoporosis model, where transgenic induction of the osteoclast inducer Rankl resulted in excessive osteoclast formation and osteoporotic bone lesions. We showed that under Rankl+ conditions, osteoblasts strongly upregulated transcription of the chemokine Cxcl9l. This chemokine in turn led to osteoclast recruitment and differentiation at bone matrix and excessive bone resorption. Mutations in cxcl9l and its cognate receptor cxcr3.2, or treatment with Cxcr3.2 antagonists prevented osteoclast formation and protected medaka bone form osteoporotic insult. To validate a possible association between the CXCL9 ortholog and osteoporosis or fracture risk in humans, we next conducted a matched caseâ€“control study nested in the prospective, population-based Singapore Chinese Health Study. This study included 55 men and 119 women who had experienced a hip fracture with an average of 6.3 years after their blood was collected. Participants were matched individually to controls who did not develop hip fractures. We observed higher blood levels of CXCL9 in the pre-fracture blood samples of men with subsequent hip fractures compared with their non-fracture controls. Surprisingly, no such differences were seen in women. This suggests that elevated CXCL9 levels affect older men and women differently, likely due to changes in sex hormone levels during aging. These findings open the possibility that early interventions targeting CXCL9-CXCR3 signalling could be beneficial in preventing hip fractures in older men.

**Dissecting the regulation of social behaviors in cichlid fishes using gene editing tools**

**Scott Juntti** (University of Maryland)
Cheng-Yu Li

The >2000 species of cichlid fish exhibit a wide variety of phenotypes in morphology, physiology, and behavior. However, how the genome encodes the information for distinct developmental and functional programs is still unclear. To address this question, we use Astatotilapia burtoni cichlids as a study species, a species with a rich ethological tradition and genetic sequencing resources. We develop and improve the tools to modify genetic sequences, enabling researchers to understand how specific genes control the form and function of the body. We have developed experimental pipelines that improved injections and editing efficiency by targeting the gene Tyrosinase. Our protocols have enabled us to generate mutants for >20 genes to permit the study of hormone signaling, pheromonal communication, and more.

**Foundational building blocks for stable genetic modification of sea urchins.**

**Amro Hamdoun** (University of California San Diego)

The utility of aquatic models depends on the effective application of stable genetic manipulation methods. Sea urchins are one of the oldest and most widely studied marine invertebrate models. However, they have been bottlenecked by the absence of lab strains or stable genetic lines. Here I will report on progress towards building a fully genetically-enabled sea urchin, using *Lytechinus pictus.* Through advances in research-scale mariculture, molecular genetics of urchins, and semi-automated high content imaging we are systematically establishing the necessary toolkit and resources necessary to remove this bottleneck. As proof of concept, we have generated homozygous knockout mutants of the sea urchin homolog of the major drug transporter ABCB1. We are now working to establish modular founder lines for cell and stage-specific mutagenesis, fluorescent reporter expression, and active genetics. The advances in this species represent a major step towards more sophisticated genetic manipulation of the sea urchin and the establishment of reproducible sea urchin lines.

**Genetic and Developmental factors contributing to eye development and degeneration in A. mexicanus**

**Johanna Kowalko** (Lehigh University)
Itzel Sifuentes-Romero, Charlotte Mullinicks, Devin Shennard, Robert Kozol, Brooke Pileggi, Rianna Ambosie, Erik Duboue, Jeffrey Trimarchi

Each organ in the body assumes a specific shape during development that is appropriate to its function. For example, formation of the spherical eye is critical to normal vision, and developmental deviations that alter this form result in structural eye defects in humans including anophthalmia, microphthalmia, or colobomas, which often result in reduction or loss of vision. Understanding the effects of the naturally occurring genetic variants, that underlie structural eye defects, on cellular behaviors during eye development is critical to understanding eye morphogenesis and how deviations to this process that lead to human disorders occur. Through studying the natural variation in eye morphogenesis, we aim to identify alterations to cellular behaviors that lead to morphogenesis defects in humans and other vertebrates. To do so, we utilize an emerging model system, the blind Mexican tetra *Astyanax mexicanus*. *A. mexicanus* is a small freshwater fish that exists in two forms, a seeing surface-dwelling form and a blind, cave-dwelling form. A. mexicanus cavefish eye development is characterized by a number of differences compared to A. mexicanus surface fish, many of which mirror structural eye defects found in humans. Through a combination of gene editing and live-imaging approaches, we have assessed a number of genes for a functional role in A. mexicanus eye development. Further, we have found that eye regression in cavefish contributes to alterations in the morphology of multiple regions of the brain. Together, this work provides insight into understanding what genes contribute to naturally occurring genetic differences in eye morphogenesis. Further, because the process of eye morphogenesis is highly conserved between species, these studies may further our understanding how structural eye defects develop in other vertebrate species, including humans.

**Dr. Frog will see you now: How *Xenopus* are moving back towards medicine by modelling human genetic diseases**

**Sian Martin** (European Xenopus Resource Centre)
Annie Godwin

*Xenopus* are established, powerful experimental tools. Only since the advent of nuclease-based gene editing have they become a potent genetic model. *X. tropicalis* is now being widely used in the diagnosis and study of rare diseases.
DNA sequencing and computational analysis has completely transformed our understanding of the genetic basis of disease. Despite this, many sufferers of genetic diseases do not get a diagnosis for some years (5.5 is the UK average) if at all, often with disastrous health consequences. Thus, gene function studies are a key tool to support pathogenicity classification and diagnostics from mountains of sequence data. By mimicking human gene variants in model organisms, functional data can be generated to test ex vivo predictions about gene function and disease causality. Such data typically demonstrate the disease mechanism including whole organism effects, tissue-specific pathogenicity, and identification of unique cellular and molecular mechanisms, information that is often very difficult or impossible to extrapolate from small cohorts. The large number of synchronous, robust and easily manipulated embryos from *Xenopus* means that they are a strong model in this experimental space. In particular, they are morphologically similar to mammals and are highly syntenic with them.
Here we describe the successful pipeline generating large numbers of F0 animals with mutations mimicking human diseases that has been used to support clinicians dealing with RGDs. We discuss the phenotyping methodology that we have applied in the pipeline, such as Micro CT and cutting edge behavioral assays allowing us to screen VUS in a UK Neurodevelopmental Disorder Cohort: phenotypes such as altered locomotion and anxiety. Finally, we summarize our work towards generating animals with precise insertions and single base changes with a view to analyzing all classes of VUS in *Xenopus*.

**Out of the sea and into the well: Developing models for the evolution of venom in marine mollusks**

**Mandë Holford** (Hunter College/AMNH)
James Parziale, Praveena Naidu, Favour Achimba, Gist Croft, Tessa Montague, Caroline Albertin

Venom is a molecular innovation. Collectively venoms are a model for investigating genotype and phenotype characteristics involved in the origin and evolution of complex adaptive traits. To reveal the function and form of venom as a complex trait, we need to be able to manipulate its genetic regulation, expression, and function on a molecular level. The only current cell model established for venomous marine snail species is limited to primary cultures from *Conus bilious*. *C. bilious* primary cultures survive for only two weeks before significant cell death, requiring optimization to be broadly utilized. With no reliable snail venom sample source outside of the animal itself, there is a bottleneck preventing the investigation of in vivo venom production and the evolutionary molecular mechanisms that drive the production and function of venom peptides. Given the immense potential of peptides from venomous organisms as molecular probes and therapeutics, it is essential to develop model systems from which we can effectively manipulate venom peptide expression and production.

**Zebrafish models of genetic epilepsies – Opportunities for translation**

**Annapurna Poduri** (Harvard Medical School)

Zebrafish have long been recognized as excellent models of human neurodevelopment. In recent years, larval zebrafish models of genetic epilepsies have recapitulated key features of early onset epilepsies, including seizures and hyperexcitability as measured by electrophysiological disturbances. This talk will review a key proof-of-principle example of a genetic model of epilepsy (modeling SCN1A-related epilepsy) leading to direct translation into the clinical realm and then outline a path forward for many types of genetic epilepsy that are amenable to modeling in zebrafish.

## Lysosomal signaling in microglia and Alzheimer's disease

**Harini Iyer** (Stanford University)
William Talbot

Genome wide association studies of Alzheimer's disease (AD) patient mutations strongly implicate immune pathways in disease onset or progression. In the healthy brain, microglia, primary immune cells of the brain, ensure nervous system well-being and function by eliminating dying cells, pruning synapses, and orchestrating appropriate immune responses. Multiple lines of evidence indicate that two essential microglial processes - lysosomal activity and inflammatory response - are aberrantly activated in AD. How these microglial processes, critical for brain development and function, become dysfunctional in the context of aging or disease remains largely unexamined. I propose to study lysosomal and immune responses of microglia in vivo using zebrafish in light of numerous observations showing that microglia cultured in vitro rapidly lose their identity and display aberrant expression of disease-associated genes. I have previously defined a lysosomal network centered around the key lysosomal transcription factors Tfeb and Tfe3. Using RNA-Sequencing of macrophages overexpressing Tfeb and Tfe3, I have uncovered the lysosomal targets of these transcription factors in the macrophage lineage. I find that many lysosomal targets of Tfeb and Tfe3 are dysregulated in AD and my ongoing experiments will reveal mechanisms through which aberrant Tfeb and Tfe3 activity contributes to the pathology in AD. In parallel, I am leveraging the experimental amenability of zebrafish for performing large-scale CRISPR screens to study genes implicated in AD. I have identified zebrafish homologs of genes mutated in AD, prioritized them based on microglial expression or lysosomal function, and performed a CRISPR mutagenesis screen. My preliminary data confirm that indeed many of these AD risk-associated genes function in microglia. Collectively, my research will bridge the gap between genomic resources available for AD and molecular mechanisms underlying the pathology of this devastating disease.

# Keynote Lecture

## Connecting aquatic models to human disease

**John H. Postlethwait**
Institute of Neuroscience, University of Oregon

Aquatic models have enormous potential to provide insights into the mechanisms of human disease and to suggest pathways to therapies. Aquatic models offer three especially useful strategies that involve genetic screens: forward and reverse screens, and evolutionary genetic screens. Small aquaria fish like zebrafish and medaka have led the way in forward and reverse genetic screens but rapid genome sequencing and CRISPR/Cas9 methodology have made reverse genetic manipulations accessible in many aquatic organisms. Such studies have provided models of human genetic diseases that have led to clinical trials. In evolutionary genetic screens, nature has selected genotypes that have led to phenotypes that are favorable in specific environmental contexts, some of which mimic human pathologies. Examples include retinal degeneration and metabolic adaptations in blind cavefish, anemia in icefishes, premature aging in some killifish, melanomas in hybrid *Xiphophora,* and many more. To optimize utility, aquatic medical models must meet several criteria for validation. For face validity, the model should replicate human clinical phenotypes. For construct validity, the model must reflect the cellular and molecular genetic mechanisms of the human disease. For predictive validity, the model should predict currently unknown aspects of human disease. With adequate support for bioinformatics that bridge aquatic medical models to each other and to humans, stock centers to preserve and supply useful animals, and the democratization of molecular tools, aquatic species can continue to provide important insights into human health and disease.

**About**

John H. Postlethwait is interested in the genetic, genomic, and evolutionary principles that guide animal development. He and his lab investigate several aspects of this main problem.

Genome Duplication: The evolution of gene functions in development after genome duplication, focusing on skeletal and neural development. His lab showed that the zebrafish genome was duplicated, that the duplication event was shared among teleosts, and the lab developed the concept of Subfunctionalization and Neofunctionalization to help understand the evolution of functions in duplicated genes.

MicroRNAs: The roles of microRNAs in embryonic development, including evolving miRNA functions after genome duplication and evolved functions of microRNAs at sub-zero temperatures in Antarctic waters.

Icefish: The genetic basis for the evolution of reduced skeletalization and truncated development of red blood cells in Antarctic icefish and their potential resilience to the warming and freshening of Antarctic oceans.

Sex determination: The developmental genetic basis for sex determination and sexual behaviors in zebrafish.

Dr. Postlethwait's work has been recognized with the George W. Beadle Award for his groundbreaking research that established zebrafish as a model system for vertebrate genetics. He built the first genetic map for zebrafish, which spurred the discovery and functional characterization of numerous genes involved in development, and showed that the zebrafish genome, along with that of distantly related teleost fish, had been duplicated. The Duplication-Degeneration-Complementation (DDC) model he proposed was a major conceptual advance that yielded insight into the mechanisms governing the evolutionary fate of duplicated genes.

# Lightning Talks & Posters

## Session 1 – Friday, October 7th

### Aquatic Genomics

**P1** **Comparative genomics of longevity: from rockfish, across mammals, and within humans**

**Stephen Treaster** (Harvard Medical School)
Joris Daleen, Jacob Daane, David Karasik, Matthew Harris

Longevity is a defining trait that varies dramatically across vertebrates. Knowledge of the mechanisms facilitating this trait and associated with maintenance of healthspan would have untold medical value. To decipher these mechanisms, we mined genetic variation among organisms exhibiting qualitative increases in longevity. We focused on variation among Rockfishes -- including several species succeeding up to 205 years -- as well as variation in longevity across mammals. We analyzed these datasets with TRACCER, a new tool to detect relative evolutionary rate convergence, to identify genes and pathways associating with changes in longevity. Canonical aging targets are identified, but we also uncover less orthodox genes associating with increase in lifespan. These include “flavonoid metabolism” among Rockfishes, which is likely a misnomer for aryl-hydrocarbon metabolism, and “chromatoid body” with pan-mammalian longevity, which may affect transposon suppression and maintenance of genomic stability. Using such broad conservation, we used these evolutionary gene sets to refine genomic variation associating with longevity in humans. Using the rockfish as a filter, we were able to remove the statistical burden of genome-wide hypothesis testing and we identified significant signals for genes and gene sets associating with human longevity. Intriguingly, we identify SNPs in the “flavonoid (aryl-hydrocarbon) metabolism” gene set as significantly associated with human survival to the 99th percentile. This indicates a conserved mechanism underlying the evolution of longevity in both rockfish and humans. The conservation among vertebrates empowers us to leverage the advantages of each dataset: the convergence of exceptional longevity in rockfish, the diversity of longevities across mammals, and the large sample sizes of human populations. Intersection of these approaches produces strong candidates for intervention to modulate aging

**P3** **Cyclostome genomes: Changes in form and function over eons and across development**

**Kaan Eşkut** (University of Kentucky)
Nataliya Timoshevskaya, Kaan Eşkut

Lampreys and hagfish are living representatives of a lineage (Agnatha, Cyclostomata: jawless vertebrates) that diverged from the common ancestor of all other living vertebrates (Gnathostomata: jawed vertebrates) more than 500 million years ago. Advances in sequencing and assembly methods are allowing the development of accurate chromosome-scale assemblies for an increasing number of cyclostome genomes. These assemblies improve our ability to resolve ancient evolutionary events that define the gene content and chromosomal structure of living vertebrate taxa.
Additionally, both lamprey and hagfish possess an exceptional mode of gene silencing, wherein specific portions of the genome are removed from most cell lineages during early development and retained only in the germline (known as programmed DNA loss). These germline-specific regions present particularly challenging targets for genome assembly due

to an enrichment of repeats and other complex sequence structures. Improved assemblies of these regions have revealed roles of ancient and recent segmental duplication from somatically retained chromosomes, intrachromosomal duplication and adaptive evolution of in shaping the content and function of eliminated sequences.
Subcellular structures that are formed during programmed DNA loss mimic features associated catastrophic DNA damage in human but are tightly regulated as a feature of normal development in sea lamprey. The ability to produce large numbers of synchronously developing embryos by in vitro fertilization has facilitated studies of the mechanisms of DNA loss.

## How to Make a Research Organism

### P5 A computational analysis of hybrid genome assembly strategies

**Joseph Walewski** (Connecticut College)

Advances in genomic sequencing technology have allowed for larger and more complex genomes to be assembled. However, for large genomes (such as salamanders and lungfish) each sequencing effort is still a notable investment; this, combined with the multiple options available for both genomic reads and software to use, motivated us to compare the possible options in order to generate the most accurate and complete genome assembly per dollar. We obtained reference genomes and sequencing reads (Illumina and Pacbio reads) from previous studies for three common model organisms (*A. thaliana, D.rerio, H. sapiens*) and the Iberian ribbed newt (*P. waltl*, so far the only newt to have its genome sequenced) and used these data to generate a variety of short and long simulated read datasets of varying coverage. Replicates of each coverage were generated, and all read combinations possible for each organism were assembled twice: once using a long read heavy strategy (implemented with FMLRC2), and once with a short read heavy strategy (implemented with LongStitch). Assemblies were assessed for percent of original genome recovered and accuracy.
While the choice of the ideal assembly strategy is dependent on many factors, in general LongStitch is able to more efficiently assemble genomes when short reads are relatively more common, and FMLRC2 is able to more efficiently assemble genomes when using a disproportionate number of long reads. For assemblies generated with approximately equal numbers of short and long reads FMLRC is able to deliver a significantly more complete genome assembly with only slightly more errors.
As the genome is the starting point for the central dogma of molecular biology, knowing the most effective way to assemble genomes can allow for a better understanding of all aquatic organisms, significantly aiding in both basic research and targeted studies for human disease, other biotechnologies, ecological studies, and many other fields of science.

### P7 Understanding sex determination in African clawed frogs (*Xenopus*)

**Sarah Burton** (Marine Biological Laboratory)
Lindsey Kukoly, Caroline Cauret, Danielle C Jordan, Ben J Evans, Marko Horb

In amphibians there is great diversity among species in the genetic mechanisms by which sex determination is achieved. For example, considerable variation exists in whether males or females are heterogametic (and thus whether there are XY/XX or ZW/ZZ sex chromosomes). In African clawed frogs (*Xenopus*), all known species have female heterogamy, however the location of the sex chromosomes varies significantly. *Xenopus*

species lack diverged sex chromosomes and instead possess sex determining loci located on different autosomes. Most *Xenopus* species display a ZZ/ZW system, where the female is the heterogametic sex. Within the *Xenopus* genus, we focused on two species: *X. laevis* and *X. tropicalis*. *X. laevis* are tetraploids, which possess L and S allo-alleles. The sex determining locus for this species has been identified as W chromosome and is located on chromosome 2L. Located within this region, the gene dm-w, determines female sex by inhibiting the expression of dmrt1(which is responsible for male sex determination). Comparatively, *X. tropicalis* is a diploid organism, where chromosome 7 is believed to possess the sex determining loci. *X. tropicalis* lacks dm-w altogether and it is unknown if there is a single sex determining gene responsible for the female sex in this species. I will present our efforts of using CRISPR-Cas9 techniques to study the mechanistic aspects of how genetic sex determination functions and evolves in *Xenopus*. Moreover, several other Xenopus mutants have been generated (dmrt1, ccdc69w, scanw, dmw & ar) to assess their involvement in sex determination. We aim to use knockout mutants and gene expression techniques to develop a comparative framework in which to better understand how the genetic basis of sex determination systems have evolved and function within the model species, *Xenopus*.

**P9** ***Xenopus* models to study genetic function in human pathology**

**Kelsey Coppenrath** (Marine Biological Laboratory)
Andre L. P. Tavares, Nikko-Ideen, Shaidani Marcin Wlizla, Sally Moody, Marko Horb

*Xenopus* provide numerous advantages for studying gene function and development of human diseases. At present, *X. tropicalis* is known to be the only diploid species of the genus and thus is preferred for generating knockout lines. Mutant lines are generated by performing microinjections into single cell embryos utilizing CRISPR-Cas9 bound to single guide RNA (sgRNA) for targeted binding. Recently we characterized our null mutant line six1 in *X. tropicalis*, which is associated with the human disease Branchio-oto-renal (BOR) syndrome. Mutations induced in the protein-protein interacting domain (SD) result in tadpoles with limited expression patterns of placode genes and craniofacial defects compared to six1 heterozygous tadpoles. Other developing mutant *X. tropicalis* lines include eya1, sf3b4, spaca4, and tspan8. Despite its allotetraploid distinction, certain genes are knockout candidates in *X. laevis*. Existing human case studies illustrate pax7 involvement in progressive congenital myopathy with scoliosis (MYOSCO). Preliminary data in *X. laevis* show double knockout of pax7 resulting in severe edemas and mortality, potentially due to its involvement in satellite cells. However, further analysis will be required to assess the gene function and expression of pax7 in *X. laevis*. Additionally, several genes, including foxc2, wt1, pkhd1, and pkd1 are being examined to understand what roles they contribute to kidney development. Here we present our work in generating knockout mutant lines to study a disease phenotype in various tissues.

**P11** **Single-cell RNA-sequencing the hypothalamus of the social cichlid *Astatotilapia burtoni***

**Andrew Hoadley** (Department of Psychology, University of Houston)
Micah Castillo, Preethi Gunaratne, Beau Alward

The cichlid fish *Astatotilapia burtoni* has a complex, but well-characterized social system, which has supported decades of research into its behavior and neurobiology. Additionally, modern functional genetics tools like CRISPR/cas9 gene editing have been optimized for use in this species. A. burtoni has been useful in elucidating key principles of the molecular

and neural mechanisms of social behaviors, including those with relevance to behavioral health. However, we do not yet have a comprehensive understanding of the cell-types involved in these processes, or molecular characterization of those cells that would facilitate a deeper understanding of the control of brain and behavior. To further A. burtoni's development as a genetically tractable model system, we are using Single-cell RNA-sequencing (scRNA-seq) to profile cell-type specific molecular markers of the hypothalamus, a brain region conserved across vertebrates that integrates social cues and coordinates adaptive physiological and behavioral responses. These individual markers, or groups of co-expressing markers, will allow us to unambiguously identify cell types in future observational and experimental work. We present preliminary data exploring the cellular landscape of the hypothalamus using scRNA-seq. Unbiased clustering analysis has allowed us to identify at least 19 putative cell types in males and females. Our findings represent the first single-cell resolution expression analysis of A. burtoni. This dataset will help further A. burtoni as a model for investigating the neural and hormonal mechanisms of behavioral health.

## Emerging Model Systems

### P13 Intraspecific variability in maternal age effects on offspring lifespan and fecundity in *Brachionus r*otifers

**Alyssa Ligouri** (Marine Biological Laboratory)
Sovannarith Korm, Alex Profetto, Emily Richters, Kristin Gribble

Across diverse taxa including humans, offspring from older mothers have been observed to have decreased lifespan and fitness. However, little is known about intraspecific variability in the magnitude of maternal age effects, or whether these effects persist across multiple generations. Monogonont rotifers are cosmopolitan zooplankton that are an emerging model system for research on aging and maternal effects due to their short lifespan, direct development, and ease of lab culture. We compared maternal age effects among four strains from the *Brachionus plicatilis* species complex. In two strains, we characterized the effects of advanced maternal age across three generations. In life table experiments for each strain, we measured lifespan, reproductive schedule, and lifetime reproductive output of mothers and of offspring produced by young (3-d-old), middle-aged (6-d-old), and old (9 – 11-d-old) mothers. Our experiments revealed unexpected variability among strains in the magnitude and direction of maternal age effects on offspring life history traits. Two strains with the longest maternal lifespans (~ 17 days) displayed stark differences in the strength of maternal age effects. In one strain, there was no effect of maternal age on offspring survivorship, and offspring from older mothers had higher fecundity early in life. In the other, we observed a strong maternal age effect on survivorship: offspring from young mothers lived 3.5 days longer than offspring from old mothers, on average. We will investigate the mechanisms driving these differences in maternal age effects in the future. Maternal age effects on lifetime fecundity persisted in old mother lines for three generations but were reversible within one generation of switching to young maternal age. These results demonstrate the need to investigate a diversity of strains and populations within model species to better understand the ubiquity of maternal age effects and their role in aging and disease.

## P15 Effects of maternal age on offspring metabolome

**Sovannarith Korm** (Marine Biological Laboratory)
Kristin Gribble

Increasing parent age at the time of offspring birth affects offspring lifespan, fecundity, and health, but little is known about the mechanisms that cause these intergenerational effects on offspring phenotype. Mitochondrial function and metabolism decline with increasing age, and so we investigated whether this age-related dysfunction is transmitted to offspring using the monogonont rotifer *Brachionus manjavacas*. Advantages of rotifers as a model system for maternal age effects research include a continuous reproductive period, direct development without metamorphosis, relatively large investment in individual offspring, and retention of hundreds of aging-related human gene homologs. We unexpectedly found that old-mother offspring have longer lifespan and increased phototaxis, but lower fecundity and lower resistance to heat stress and mitochondrial complex I inhibition relative to young-mother offspring. We conducted targeted metabolomics to determine the age-related changes in metabolic features of mothers and in offspring from young, middle-aged, and old mothers. With increasing age, mother rotifers accumulate carnitine synthesis metabolites, including carnitine and its metabolic products, short and dicarboxylic chain acylcarnitines. This suggests a decline in fatty acid oxidation and the tricarboxylic acid cycle in old-mother offspring. Acylcarnitines also accumulate with increasing age in young-mother offspring but remain at lower concentrations throughout life in old-mother offspring. Moreover, old-mother offspring maintain lower oxidized glutathione throughout life and high spermidine metabolites in early life relative to younger-mother offspring. These pathways have been reported to extend lifespan and improve physical functions in other organisms. Thus, these functional, phenotypic, and metabolomic studies may allow us to identify potential therapeutic targets to improve rotifer offspring lifespan, with potential for translation to human health.

## P17 Confocal imaging of zebrafish larvae reveals that glucocorticoids suppress neutrophil responses to aspergillus fungal infection

**Savini Thrikawala** (Clemson University)
Molly Anderson, Emily Rosowski

Prolonged use of glucocorticoids is a major risk factor for developing invasive aspergillosis caused by a ubiquitous fungus Aspergillus. Using a zebrafish larva-Aspergillus infection model, our goal is to identify the molecular and cellular immune mechanisms that glucocorticoids suppress to make hosts susceptible to invasive aspergillosis.
To test the effects of glucocorticoids, we microinjected Aspergillus spores into the hindbrain ventricle of zebrafish larvae, and dexamethasone was added into the larval water. Dexamethasone exposure significantly decreases infected larvae survival. To test the significance of specific cell type-mediated responses, we infected macrophage-deficient and neutrophil-deficient larvae. When exposed to dexamethasone, neutrophil-deficient larvae succumb to the infection at a higher rate than macrophage-deficient larvae, suggesting that dexamethasone predominantly suppresses neutrophil-mediated responses. We used confocal microscopy to determine the effect of dexamethasone on neutrophil-mediated fungal control. Through daily, live, confocal imaging of larvae, we find that neutrophils fail to control Aspergillus hyphal growth in the presence of dexamethasone. Larvae with mutations in irf8, which lack macrophages, but have an increased number of neutrophils, also fail to contain hyphal growth with dexamethasone exposure. Using live imaging of larvae from a NF-κB reporter line, we find that dexamethasone suppresses NF-κB activation in Aspergillus-

infected zebrafish larvae. To test the significance of NF-κB, we used a CRISPR/Cas9 system to inhibit NF-κB activation in F0 larvae. Similar to dexamethasone exposure, irf8 mutant larvae injected with guide RNAs targeting Ikkγ also fail to control Aspergillus hyphal growth and succumb to the infection. Collectively, we find that glucocorticoids suppress neutrophil control of Aspergillus hyphae by inhibiting NF-κB activation in zebrafish larvae.

**P19 The role of SRF and Myostatin in muscle glucose uptake using rainbow trout**

**Michael Addo** (University of Alabama at Birmingham)
Peggy Biga

The mechanisms associated with improving glucose uptake are targets for developing therapies for impaired glucose homeostasis observed in Type 2 diabetes. Previous studies show that decreased SRF transcriptional activity increases skeletal muscle glucose uptake. Serum Response Factor (SRF), a transcription factor, regulates the myogenic gene myostatin, which negatively regulates muscle tissue growth in mammals and decreases glucose transporter (GLUT4) expression in muscles. These studies highlight the role of SRF and myostatin in glucose uptake. Rainbow trout (Oncorhynchus mykiss) are poor glucose utilizers despite having functional insulin receptors and glucose transporters. Thus, making them a good model for studying impaired glucose homeostasis. Additionally, rainbow trout express multiple myostatin isoforms that exhibit differential regulation. Fasting alters myostatin mRNA expression but how fasting affects the truncated myostatin-2b isoform remains unknown. The myostatin-2b gene contains several SRF binding sites, and thus we hypothesize that fasting will increase myostatin expression through an SRF-dependent program. Thus, potentially decreasing glucose uptake in muscle. This study will investigate the role SRF plays in regulating muscle glucose uptake, including unraveling its effects on myostatin signaling. Data will shed light on the mechanisms of action of myogenic signaling on glucose uptake directly and will clarify how SRF- dependent myostatin signaling could serve as a marker for insulin resistance.

**P21 *Berghia stephanieae*: A new model mollusc for studying the interaction of a brain with a distributed neural network**

**Paul Katz** (University of Massachusetts Amherst)

Gastropod molluscs provide an opportunity to study the interactions between a brain and a distributed neural network that grows continuously and regenerates. In addition to a brain with large identifiable neurons, that gastropods such as *Aplysia* are well known for, they possess a peripheral neural network (PNN) consisting of peripheral ganglia and a subepithelial neural plexus (SNP) with local motor neurons, sensory neurons, and inter-neurons. We are characterizing neurons in the brain and PNN of the gastropod, *Berghia stephanieae*, an emerging model species. During post-metamorphic development, animals grew from about 500um to over a centimeter with the PNN increasing in cell number to match the expansion. The brain also increased in cell number from about 500 neurons at the early juvenile stage to over 5000 in the adult. We mapped gene expression in neurons of the adult brain and PNN using single cell transcriptomics, in situ hybridization chain reaction, and immunohistochemistry. We found that PNN neurons have similar neurotransmitter diversity to brain neurons, including small molecule transmitters such as acetylcholine, histamine, and dopamine. Neuropeptides such as conopressin, the molluscan ortholog of vasopressin/oxytocin and neuropeptide Y, the ortholog of NPF, were expressed in large identifiable brain neurons as well as hundreds of small neurons throughout the PNN. We found that the PNN supports local and coordinated movement; when the brain was removed,

the body still performed some movements. The PNN in *Berghia* is likely related to the peripheral network that controls the semi-autonomous arm movements in a more complicated mollusc, the octopus. In many ways, the gastropod PNN also resembles the vertebrate enteric nervous system (ENS). Thus, *Berghia* could provide general insights into network organization and function that could be medically relevant to humans.

**P23 Nidovirus infection patterns in California sea hares, *Aplysia californica***

**Michael Schmale** (University of Miami)
Dayana Vidal, Lynne Fieber, Benjamin Neuman, Nicholas Kron

*Aplysia californica* has been widely used in neuroscience research for over 50 years. Animals are either obtained from the wild by collectors or from laboratory reared populations at the National Resource for Aplysia. Two recent studies documented the genome of a novel, very large (34 kb), nidovirus in RNA sequence databases from A. californica. The goal of the present study was to document the distribution of this virus, termed Aplysia Abyssovirus (AAbV), in hatchery reared as well as wild collected animals and to investigate viral loads and patterns of subgenomic transcripts. We found that this virus is widespread in both wild and hatchery reared animals and that viral loads can reach extraordinary levels in apparently healthy animals. The tendency of AAbV to reach highest levels in the nervous system is especially surprising considering the lack of obvious pathology in such animals. This raised the question of the replication status of these viral genomes, particularly at high levels of infection. We found that AAbV viral transcripts do not exhibit any evidence of splicing that would produce subgenomic RNAs consistent with predicted open reading frames that are typically observed in all previously described nidoviruses. In particular, there is no evidence of any ORF2 subgenomic transcripts. Instead there appear to be a very wide variety of splice sites, none of which seem to be canonical or individually common. Finally, and perhaps most unusually, in animals with high viral loads the vast majority of transcripts are negative sense, a scenario not consistent with a productive viral infection as AAbV is a positive sense RNA virus. Thus, we propose that animals may be able to survive high viral loads as measured by RNA levels due to the lack of functional replication of most or all of the viral sequences detected in adult animals. AAbV may serve as a new model of chronic viral infection by a virus in the same order (albeit a different family) as coronaviruses.

**P25 Investigating the mechanisms behind CPEB2-mediated regulation of spermatogenesis**

**Fanghemei Zhang** (University of Massachusetts Boston)
Junichi Tasaki, Labib Rouhana

Germline development across the animal kingdom requires highly conserved molecular mechanisms. Sexual Planarians are hermaphroditic organisms of the phylum Lophotrochozoa that continuously produce both sperm and eggs. Gene knockdown via RNA interference can be applied to juvenile or adult planarians by simply feeding dsRNA, and germline development phenotypes can be observed within weeks. Therefore, planarians are a great model for the identification of genes involved in animal germline development. Cytoplasmic Polyadenylation Element Binding proteins (CPEBs) bind to specific mRNAs and regulate translation. Smed-CPEB2 is a CPEB homolog that is expressed in the testes and the central nervous system of the planarian Schmidtea mediterranea. Smed-CPEB2 is required for spermatogenesis as well as for overall reproductive maturation through the maintenance of Neuropeptide Y-8 levels. Details about the direct molecular mechanisms by which CPEB2 regulates spermatogenesis or neuropeptide levels remain unknown. We

identified 67 potential protein partners of CPEB2 by performing co-immunoprecipitation and mass spectrometry. We hypothesize that some of these candidate partner proteins facilitate Smed-CPEB2 function and are therefore essential for sperm development. Thus far, we have found that RNAi-mediated knockdown of eight different candidate CPEB2 partners results in failure to complete spermatogenesis. Detailed phenotypic analyses to identify which stage of spermatogenesis correlates with each protein are ongoing. In addition, efforts to identify mRNAs regulated by Smed-CPEB2 will also shed light on the molecular mechanisms by which CPEB2 controls sperm development.

**P27** **Exploring the environmental and molecular underpinnings of reproductive disorders in an ecologically relevant model, the American alligator**

**Christopher Smaga** (University of Georgia)
Samantha Bock, Mathew Hale, Benjamin Parrott

Reproductive disorders affecting ovarian function are a common contributor to global infertility and in most cases, are highly idiopathic with complex genetic and environmental origins. American alligators are long-lived, oviparous reptiles whose plasticity in sex determination, high site fidelity, and propensity to bioaccumulate contaminants make them a unique model for investigating environmental influences on reproductive health. The alligator population at Lake Apopka, Florida is exposed to a suite of endocrine disrupting contaminants (EDCs) and displays disrupted ovarian phenotypes that resemble ovarian disorders in humans, such as primary ovarian insufficiency (POI), which affects 1-2% of women. Indeed, juvenile alligators from Lake Apopka display a dramatic reduction in the number of late-stage ovarian follicles accompanied by broad scale transcriptional changes, neither of which are rescued by exogenous gonadotropin treatments. Many of the EDCs present in Lake Apopka can activate nuclear estrogen receptors and previous work revealed that treating embryos from a reference site with estrogen just prior to ovarian differentiation and estrogen synthesis recapitulates the transcriptomic and histological perturbations observed in alligators from this site. These findings suggest that disrupted timing of developmental estrogen signaling may be a critical event in the etiology of POI-like phenotypes. However, we lack a basic understanding of the molecular pathways that are differentially impacted by the timing of embryonic estrogen signaling. To test this, we compare ovarian transcriptomes of alligator embryos treated with estrogen or vehicle at two important developmental timepoints and relate them to differences seen previously at Lake Apopka. Our results provide insight into how timing impacts the molecular pathways influenced by estrogen, and its importance for deciphering mechanisms of EDC driven reproductive disorders.

**P29** **Groundwork for systematic phenotyping across animal models using X-ray histotomography**

**Rachelle Saint-Fort** (Penn State College of Medicine)
Khai C. Ang, Mee Siing Ngu, Daniel J. Vanselow, Keith C. Cheng

The level of understanding a gene's function or a chemical's effects on an organism is determined by the depth of phenotyping. Ideally, anatomic phenotyping can be done across all cell types and organs in a whole organism and include assessments of histopathological change that allows the definition of cellular mechanisms of disease. To accomplish whole-organism phenotyping requires sub-micron voxel resolution to detect the micron-scale features characteristic of pathological features, and sufficient field-of-view at that resolution to cover the entire organism. To set the stage for whole-organism, quantitative tissue

phenotyping, we created a 3D form of histology termed X-ray histotomography, which produces high-resolution images of centimeter-scale organisms at 0.5 micron voxel resolution. Our imaging and segmentation pipeline for Daphnia and zebrafish, enables us to expand our work to include amphibian models, particularly wild-type axolotl larvae (stage 44), imaged at the Berkeley Advanced Light Source beamline, generating a 1.4TB reconstructed image file at 32bits for a 9 mm by 2.5 mm sample. Using these three aquatic model systems, we propose to find shared features of common cell and tissue types despite specific-organismal differences. The envisioned outcome will result in computationally derived characterizations of normal cell morphology across the three models, including defining the statistical normal distribution of cell and organ volume, shape, texture, and the measured relationships between cell types. The digital data generated from these aquatic samples holds enormous potential especially when integrated with corresponding molecular data to further our understanding of the origins of human disease.

## Stem Cells & Regeneration

### P31 Photodegradable hydrogels for dynamic tuning of physical and chemical properties

**Sam Norris** (University of California Los Angeles)
Andrea Kasko

While developmental biologists have extensively studied signaling factors, gene expression and other components governing early tissue morphogenesis, we still do not have a full picture of how these signaling cascades result in spatial and temporal differences in tissue physical properties and the resulting mechanical forces required to shape tissue structure. As cells pull on their surrounding environment, intracellular tension is generated, the magnitude of which typically scales with the stiffness of the surrounding extracellular matrix (ECM). Cells convert these physical forces into chemical and electrochemical signals through a variety of pathways and mechanisms. In order to study how biophysical factors, influence cell and tissue fate, researchers have utilized hydrogel since their physical properties can be controllably tuned. To study cell response to dynamic environments, researchers have developed novel hydrogel chemistries that allow for hydrogel properties to be tuned in both time and space, typically through the use of light. In this work, we develop novel photodegradable materials, find solutions to better characterize their behavior, and expand the techniques necessary for their successful use in developmental biology. To better mimic three-dimensional cellular environments, we successfully synthesize photodegradable protein-based gels and showcase their applicability towards three-dimensional cell culture. Next, we synthesize and fabricate photodegradable polyacrylamide gels and explore cell response to both changes in cell binding domain and dynamic softening of the underlying matrix. Finally, we develop the application of mask-less photolithography for photodegradable hydrogels to pattern grayscale stiffness patterns into photodegradable hydrogels in a highly controlled fashion with sub-micron resolutions. Through these developments, we expand our ability to test cell behavior in spatially and temporally heterogeneous environments.

**P33** **The role of YAP and TAZ in neural stemness and regeneration**

**Mirjana Malnar Črnigoj** (Paracelsus Medical University)
Jan Pruszak

Hippo-YAP/TAZ signaling is involved in organ size, proliferation, and stem cell niche control throughout the human body. In neurogenic niches, it has been shown to impact proliferation, differentiation, and apoptosis of neural precursor cells, which enable physiological neural repair through active neurogenesis. Therefore, a better insight into this signaling pathway`s role in neurogenesis could provide important tools for harnessing intrinsic stem cell potential for regenerative purposes.
The downstream effectors of the Hippo pathway, yes-associated protein-1 (YAP) and its paralog WW domain-containing transcription regulator 1 (WWTR1 or TAZ), act as transcriptional co-activators of genes involved in cell fate determination. Both YAP and TAZ are downregulated during neuronal differentiation, whereas their overexpression can convert multiple cells, including neurons, to their respective lineage-restricted somatic stem cells, showing promising possibilities for stem cell therapy. YAP and TAZ have largely been considered as interchangeable; however, they can maintain a stem or progenitor state or promote differentiation in different contexts, showing distinct roles during embryogenesis and cell fate specification. Moreover, their co-transcriptional activity is in part regulated via nucleocytoplasmic localization and potentially also different protein binding partners. Furthermore, there are different (also neural-specific) isoforms of YAP and TAZ and knowing their expression profiles and potential roles in neural differentiation could give crucial insight into human neurogenesis.
We aim to elucidate the role of YAP and TAZ and their isoforms in human neurogenesis using state-of-the-art in vitro models and approaches (e.g. CRISPR-editing, stem cell-derived neural lineages, brain organoids). A better understanding of YAP and TAZ in neurogenesis could lead to novel paradigms in harnessing neural regenerative potential in stem cell therapy.

**P35** **Use of IPSC-derived neural organoids from an Alzheimer's disease patient for the study of novel PSEN1 T119I mutation**

**Luciana Isaja** (Instituto de Neurociencias, FLENI-CONICET)
Mariela Marazita, María Soledad Rodríguez Varela, Sofía Mucci, Tatiana Itzcovich, Patricio Chrem-Méndez, Sofía Luján Ferriol-Laffouillere, Ricardo Allegri, Horacio Martinetto, Gustavo Emilio Sevlever, María Elida Scassa, Ezequiel Ignacio Surace, Leonardo Romorini

Presenilin-1 (PSEN1) gene mutations are the most common cause of familial Alzheimer's disease (fAD). Previously, our group generated a human induced pluripotent stem cell (hiPSC) line (FLENIi001-A) from a male carrier of the heterozygous PSEN1 p.T119I variant. To functionally validate the PSEN1 (p.T119I) variant, we generated a 3D brain organoid (BO) model to study hallmarks of AD, such as AÎ² deposition and tau hyperphosphorylation. BOs were generated from FLENIi001-A and control hiPSCs following a published protocol. Briefly, hiPSCs were first single-cell detached with Accutase and plated in 96-well ultra-low attachment plates at 21000 cells/well in human ESC medium and fiber solution. At day 6, medium was replaced by neural induction medium (DMEM/F12, N2 and heparin). On day 11, BOs were embedded in Matrigel and cultured in ultra-low attachment p60 plates (10-16 BOs/well) in differentiation medium (50% DMEM/F12-50% Neurobasal, N2, B27 without vitamin A and insulin). After 4-5 days, BOs were transferred to differentiation medium (with B27 with vitamin A) and plates swirled in an orbital shaker. Medium was replaced every 5

days and from day 40 Matrigel was added in every medium change. BOs were fixed at days 30, 60 and 90 and immunostained for neural and cortical markers. Day 90 BOs were inmunostained for neurodegeneration markers (AÎ² plaques and phospho-Tau; Thr 205). FLENIi001-A BOs showed expected morphology patterns such as neuroepithelial buds and smooth cortical lobes. Also, expression of neural (PAX-6, MAP-2, NeuN, and TUJ-1) and cortical (CTIP2 and TBR1) markers was detected in 60- and 90-days BOs. These BOs exhibited: increased AÎ² and p-Thr-205 tau accumulation when compared to wild-type counterparts. Overall, we successfully obtained BOs from hiPSCs line FLENIi001-A carrying the AD PSEN1 p.T119I genotype that showed AD pathological features. These preliminary results add further functional evidence of pathogenicity for this variant.

**P37 The Impact of manganese on glutamate excitotoxicity in Alzheimer's disease model**

**Hyunjin Kin** (Purdue University)
Anke Tukker, Fiona Harrison, Aaron B. Bowman

Alzheimer's disease (AD) is the most common cause of dementia. The pathological alterations that occur at the molecular/cellular level begin many years before disease onset and the majority of AD cases cannot be attributed to a single gene. This supports the involvement of chronic environmental risk factors in AD etiology. Manganese (Mn) is an essential metal widely present in the environment and in excess is a neurotoxin. As the brain's major excitatory neurotransmitter, glutamate homeostasis is under tight regulation. Under physiological conditions, glutamate is removed from the synapse primarily by astrocytes to prevent excitotoxicity. Also, acute exposure to high levels of Mn is reported to inhibit glutamate uptake. This line of evidence suggests a potential pathological relationship between Mn neurotoxicity and AD pathology via glutamate excitotoxicity. However, interactions between chronic environmentally relevant Mn exposures and AD genetics are unknown. Taken together, we hypothesize that individuals with genetic risk factors for AD have enhanced susceptibility to glutamate dysfunction in response to chronic Mn exposure. To address this hypothesis, we utilize cortical glutamatergic neurons and astrocytes generated from human induced pluripotent stem cells derived from healthy and AD patients. Cells were cultured for approximately 100 days and exposed to environmentally relevant levels of Mn (0.5 or 5 Î¼M vs control) for up to 40 days. Following exposure, cells were treated with 14C-radiolabeled glutamate to quantify glutamate uptake. Our results suggest the presence of an AD genotype effect wherein, we observed a significant decrease in glutamate uptake in AD patient-derived neurons/astrocytes compared to control cells. Further, single cell RNA-sequencing and pathway analyses revealed alterations in key metabolic pathways. Additional efforts are being conducted to address functional alterations via micro-electrode arrays.

## Session 2 – Saturday, October 8th

### Developmental Disorders

**P2** **Linking mitochondria and cell proliferation in the developing brain**

**Maggie Kettelberger** (William & Mary)
Martin Feng, Olivia Frankel, Adithi Ramakrishnan, Jennifer Bestman

Radial glial neural progenitor cells (NPCs) are the heterogeneous proliferating stem cell population that gives rise to all cells in the brain. Early on, NPCs are largely symmetrically dividing, progenitor pool-expanding cells, but as development continues the NPCs begin to asymmetrically divide to produce neurons. Intrinsic and extrinsic cues guide this irreversible and critical fate switch. If regulated inappropriately, a premature shift to neurogenesis limits brain development and contributes to neurodevelopmental disorders. Although proliferating cells do not rely on mitochondria for ATP, mitochondria are abundant in these cells. The diverse cellular functions of mitochondria place them at the intersection of the intrinsic and extrinsic cues that guide the fate of NPCs. We use in vivo time-lapse confocal microscopy to image fluorescent protein-labeled NPCs in the optic tectum of albino *Xenopus laevis* tadpoles. This approach preserves the surrounding neural circuitry of the developing visual system and allows us to monitor and track the NPCs, their mitochondria, and their progeny over days. When all mitochondria in the NPCs are labeled with mito-localized GFP or subpopulations with photo-activatable GFP, we find that their distribution is biased as the NPCs prepare for symmetric cell division, suggesting that the position of the mitochondria may forecast or play a role in the type of division. Our data also show that interfering with mitochondria through the delivery of the antisense morpholino against metabolic regulator peroxisome proliferator-activated receptor Gamma co-activator 1a (PGC-1a),) a transcription factor that regulates mitochondrial biogenesis, limits neurogenesis. Together our data suggest that mitochondria within NPCs contribute to their fate.

**P4** **Identification of ancestral gnathostome Gli3 enhancers with activity in mammals**

**Shahid Ali** (The University of Chicago)
Shahid Ali, Muhammad Abrar, Irfan Hussain, Fatima Batool, Rabail Zehra, Hizran Khatoon, Axel Visel, Neil H. Shubin, Marco Osterwalder, Amir Ali Abbasi

Abnormal expression of the transcriptional regulator and hedgehog (Hh) signaling pathway effector GLI3 is known to trigger congenital disease, most frequently in the central nervous system and the limbs. Accurate delineation of the genomic cis-regulatory landscape controlling GLI3 transcription during embryonic development is critical for the interpretation of non-coding variants associated with congenital defects.
Results: Here we employed a comparative genomic analysis on fish species with a slow rate of molecular evolution to identify seven previously unknown conserved noncoding elements (CNEs) in Gli3 intronic intervals (CNE15-21). Transgenic assays in zebrafish revealed that a majority of these elements drive enhancer activity in fins, CNS, or heart. Comparative functional dissection of a previously identified intronic ancestral CNE with limb enhancer function (CNE14/hs1586) indicates co-option of limb specificity from other tissues prior to the divergence of amniotes and lobe-finned fish. Finally, intersection of these CNEs with human disease-associated SNPs identifies CNE15 as a putative craniofacial enhancer with conserved activity in vertebrates and potentially affected by mutations associated with human facial morphology.

Conclusion: These results uncover a cluster of intronic Gli3 enhancers which arose in the common ancestor of gnathostomes and whose components were likely gradually modified in other species through sequence diversification.

## Cancer & Aging

### P6 Identification of *smarcad1a* as a new tumor suppressor gene of malignant peripheral nerve sheath tumors in zebrafish

**GuangJun Zhang** (Purdue University)
Han Han, Guangzhen Jiang, Rashmi Kumari, Martin Silic

Malignant peripheral nerve sheath tumors (MPNSTs) are relatively common sarcomas generally found along the nerves and originate from Schwann cells. The prognosis for this type of malignancy is relatively poor due to complicated genetic alterations and the lack of specific targeted therapy. Etiologically, the genetic mutations contributing to MPNSTs remain largely unknown except for the NF1 and TP53 genes. Chromosome fragment 4q22-23 is frequently deleted in MPNSTs and other human tumors, suggesting tumor suppressor genes may reside in this region. Here, we provide evidence that SMARCAD1, a known chromatin remodeler, is a novel tumor suppressor gene in the 4q22-23. There are two human homologous smarcad1 genes (smarcad1a and smarcad1b) in zebrafish. We created zebrafish mutant fish lines for both and found that homozygotes of the two genes are viable in adulthood. Also, we showed that two smarcad1a mutations accelerated MPNST tumorigenesis. This suggests smarcad1a is a new tumor suppressor gene for MPNSTs. Moreover, we found that DNA double-strand break (DSB) repair was compromised in smarcad1a mutants. Furthermore, SMARCAD1 gene knockdown and overexpression in human cells were able to inhibit tumor growth and displayed similar DSB repair responses, suggesting proper SMARCAD1 gene expression level is critical for normal cell growth. Given that mutations of SMARCAD1 can sensitize cells to poly ADP ribose polymerase inhibitors in yeast and human cells, the identification of SMARCAD1 as a novel tumor suppressor gene might contribute to the development of new cancer therapies for MPNSTs.

### P8 Caloric restriction affects gene expression and learning capabilities during operant conditioning in *Aplysia californica*

**Eric Randolph** (University of Miami)
Lynne Fieber

Cognitive declines are commonly associated with advanced age and senescence of the nervous system. Caloric restriction (CR) as a form of diet is shown to slow cellular senescence and prolong life. As an additional side effect, aged CR animals exhibit enhanced learning capabilities when compared to their same aged counterparts fed on an unrestricted ad libitum (AL) diet. Whether learning enhancements in aged CR animals is attributable to healthier cellular aging or whether diet induces variable changes in gene expression in learning is debatable. Despite this uncertainty, comparing the transcriptional response of the brain after learning between aged AL and CR animals is unstudied. RNASeg was performed on ganglia of sibling Aplysia californica raised on AL and CR diets and then trained in an operant learning paradigm to identify an inedible food source at ages 7- (mature) and 10-months (aged) to identify transcriptional changes and how they coincided with learning. Unexpectedly, aged AL animals outperformed their younger AL siblings in identifying an inedible food source while aged CR animals did not show any change in performance

compared to their younger AL siblings. Analysis of AL animals identified 77 differentially expressed genes (DEG) at maturity and 53 DEG in age. After learning, aged AL animals showed higher transcription levels of Neuropeptide Y and its receptor NPY1R which are implicated in mimicking the effects of satiation. Furthermore, the transcription of cytoarchitecture genes STMN1 and ABRA, as well as genes associated with long-term memory such as Rab3, PIN, and EPDR1 were also increased after learning in aged AL animals. Preliminary analysis of aged CR animals after learning identified 68 DEG of which 54 are unique compared to aged animals fed AL. These genes are currently being assessed. This research may provide gene sets and pathways that can be studied in other model organisms that may aid understanding of the cognitive effects of diet and age.

**P10** **PML, PML-like Exon 9 (Plex9), and TREX1 regulate LINE retrotransposition in jawed vertebrates**

**Sabateeshan Mathavarajah** (Dalhousie University)
Kathleen Vergunst, Shelby Williams, Raymond He, Maria, Maliougina, Elias Habib, Mika, Park, Jayme Salsman, Stéphane Roy, Ingo Braasch, Andrew J. Roger, David N. Langelaan, Graham Dellaire

The PML gene is a tumor suppressor in a variety of cancers through the regulation of DNA damage repair, senescence and innate immune signaling. While PML is well-studied, there is a remaining mystery behind the function of the PML exon 9 region, which is only present in the PML-I isoform (the most highly expressed isoform). Here, we use a molecular evolution approach to better understand the potential functions of the C-terminus domain encoded by exon 9. Using comparative genomics, we identified homologs of PML in different vertebrate species. A progenitor full-length PML gene can be identified in gar, a basal ray-finned fish. However, in teleost fish like zebrafish and in salamanders, PML gene homologs are truncated and only align to exon 9 of human PML. We cloned cDNAs of gar PML and PML-like exon 9 (Plex9) proteins found in fish and orthologs of TREX1 in salamanders. Intriguingly, the Plex9 proteins resemble DEDDh exonucleases that are known to suppress LINE-1 retroelements. A role for regulating LINE-1 activity was assessed for these proteins using a clonogenic neomycin-selection based LINE-1 retrotransposition assay. When expressed in human cells, these proteins localize to the ER, cytoplasm, and nucleus. We found that gar PML, Plex9 proteins, and TREX1 orthologs potently suppress LINE-1 activity. The function of PML in LINE-1 retrotransposition is conserved in mammals as well. The loss of human PML elevates LINE-1 retrotransposition and the addback of PML or orthologs of PML suppresses this phenotype. Our findings with gar PML and anamniote Plex9 proteins suggest a so far unrecognized role for PML in the regulation of LINE-1 activity. Discovering such functions for PML dating back to the bony vertebrate ancestor may help us better understand how the PML-I isoform contributes to mammalian tumor suppression.

## Animal Reference Diets & Nutrition

**P12** **Improving rainbow trout *Oncorhynchus mykiss* growth performance through optimization of diet-epigenetic interactions**

**Khalid Freij** (University of Alabama at Birmingham)
Beth Cleveland, Peggy Biga

The aquaculture industry has vastly expanded in recent years and accounts for half of seafood consumed globally. With this expansion, sustainable shifts in aquaculture must be

made to access nutrients essential to human health in response to a decline in marine resources for aquafeeds. Along with shifts in aquafeed composition, selective breeding programs aid in eliminating mortality rates in fish populations aimed for the marketplace. This study will also inform us on parallels found in human maternal diet influence on offspring metabolic health. Rainbow trout are a staple aquaculture species and serve as a non-model organism to investigate toxicology, evolutionary biology, and nutritional programming. Understanding the impacts of nutritional programming in aquaculture species will aid in understanding the effects of broodstock nutrition on offspring growth performance via inherited epigenetic mechanisms while providing information regarding potential mechanisms of maternal effects. Therefore, this project focuses on the interactions between maternal nutrition and genetic selection utilizing rainbow trout, *Oncorhynchus mykiss*, used within the industry disease-resistant selected rainbow trout maintained by the National Center for Cool and Cold Water Aquaculture. The overall project objective includes identifying specific genes and gene pathways in offspring affected by maternal dietary intake of choline supplementation during oogenesis. To accomplish this, global and local DNA methylation patterns were analyzed in trout offspring and compared to global transcriptomic data from corresponding samples. Treatment effects on the methylome and transcriptome were analyzed to identify potential mechanisms altered by maternal choline intake and establish links between epigenetic modifications in the genome and phenotype of the offspring. Results indicate that several metabolic and tissue-specific pathways are under, at least, partial maternal regulation.

## Immunology & Microbiome

### P14 The composition of *Xiphophorus maculatus* gut microbiome is influenced by diet

**Erika Soria** (Xiphophorus Genetic Stock Center, Texas State University)
Crystal Russo, William Boswell, Markita Savage, Lindsey Sanchez, Zoltan M. Varga, Merritt Drewery, Camila Carlos-Shanley, Yuan Lu

Recent advances in microbiome studies suggest that interactions between the gut microbiota and host organs (e.g., gut-brain axis) can influence host health. These interactions can affect production of metabolites and have been associated with several diseases and disorders. Diet has been shown to affect the gut microbiome and influence the physiological baseline of research animals and data reproducibility. To control diet-induced variations, standardized diets have been developed for biomedical models such as rodents and zebrafish. Despite the use of *Xiphophorus* fish in biomedical research for decades, a reference standard diet has not been developed. In this study, we compared two diets including a commercial zebrafish diet, Gemma (GEM), and a proposed zebrafish reference diet developed by the Watts laboratory at the University of Alabama at Birmingham (WAT) to the Xiphophorus Genetic Stock Center custom diet (Control) to identify differences in the *Xiphophorus* gut microbiome. *Xiphophorus maculatus* were fed the three diets and feces collections were taken monthly between the ages of two to six months (Sample size: GEM = 25; WAT = 23; and Control = 22). Feces collections were analyzed using 16S rRNA sequencing. We observed gut microbiome alterations were driven by both diet and age, while diet exhibited a larger effect on gut microbiota composition. Our results indicate that diets developed specifically for zebrafish may not be optimal for *Xiphophorus*. Thus, a species-specific reference standard diet needs to be developed for *Xiphophorus* and potentially other aquatic model organisms.

**P16** **Critical windows of immune development in *Oryzias melastigma*, an immunotoxicology model**

**Elizabeth DiBona** (Texas A&M University-Corpus Christi)
Marissa Brown, Desirea Harder-Neely, Frauke Seemann

Prevalence of immune diseases and the role of pollutant exposure in manifestation of these diseases is a rising human health concern. The developmental origins of health and diseases (DOHaD) hypothesis that pollutant exposures during immune system development trigger immune pathologies directly addresses this concern. Periods of higher susceptibility during immune development, critical windows, must first be defined. Here we propose use of the teleost model, marine medaka (*Oryzias melastigma*), to develop a molecular timeline of immune system development in vertebrates. Changes in survival and expression of immune genes during development will provide a basis for defining critical windows. Survival was assessed via pathogen challenge using Edwardsiella piscicida. Significant changes in immune competence were seen at 7-11 days post fertilization (dpf), 3-5 days post hatching (dph), and 9-12 dph. Gene expression of immune initiators (C1q, TLR5-soluble, TLR5-membrane), mediators (MYD88), and effectors (LYZ) revealed significant changes at the same critical windows. Together organismal and molecular level changes outline three major critical windows of immune development in marine medaka which overlap with major changes in cellular immune development including migration of lymphoid progenitor cells, colonization of the thymus, T-cell maturation, and immune competence of the thymus. These cellular immune development timepoints are essential for a functioning and self-regulating immune system; alterations to normal T-cell function could result in an over- or under-active immune response. Pollutant exposures during these critical windows may impact development and maturation of T-cells leading to immune pathologies like chronic inflammation in later-life stages which is a relevant concern for human health. These critical windows will provide a useful tool to assess the developmental immunotoxicity of environmental pollutants and subsequent immune disease manifestation.

## Toxicology & Environmental Influences on Health

**P18** **The possible role of miRNA-199a in BaP-induced transgenerational bone deformities in Japanese medaka**

**Rijith Jayarajan** (Texas A&M University-Corpus Christi)
Jiezhang Mo, Alexis Trujillo, Erik Gonzalez, Frauke Seemann

Bone diseases such as osteoporosis are a major concern worldwide due to the increasing prevalence in the population. Benzo[a]pyrene (BaP), an ubiquitous environmental pollutant and known carcinogen, has been evidenced to cause bone disorders in a transgenerational manner by altering epigenetic mechanisms like microRNAs (miRNA). Transcriptome analysis of bone tissue in the ancestrally BaP-exposed F3 generation indicated a prominent role of miR-199a in the regulation of significantly enriched bone formation and oxidative stress pathways. To elucidate the function of miRNA during bone development wildtype (Orange Red) and transgenic (twist:dsred/col10a1:gfp & col10a1:gfp/osx:mCherry) freshwater medaka (*Oryzias latipes*) were assessed for calcification and cell-subpopulation level changes in response to miR-199a agonist and antagonist microinjection. This study provides a novel understanding of miRNA regulation during two critical windows during bone development. Agonists injection at the beginning of bone formation significantly reduced

bone calcification at 24-30 days post fertilization (dpf). Osteoblast subpopulation analysis showed that the fluorescence area and intensity were decreased at 30 dpf for twist rfp+ sclerotomal cells. There was a significant reduction of col10 gfp+ osteoblast progenitors at 25 dpf in response to miR-199a upregulation. Osx rfp+ premature osteoblasts also showed a significant decreased spatial distribution at 25 dpf. The expression of miR-199a and the target genes have been assessed at 25 dpf. The overall results confirm the role of miR-199a in reducing bone calcification by affecting the maturation of osteoblasts. The provided data can be used to delineate the effect of BaP exposure on the regulation of specific miRNAs. Moreover, developmental levels of miR-199a may provide useful as a potential biomarker for later life-stage bone impairment, such as osteoporosis development.

**P20 Using fish models to understand the role of the human tumor suppressor AIP (AHR-Interacting Protein) as a chemical susceptibility gene**

**Mark Hahn** (Woods Hole Oceanographic Institution)
Sibel I. Karchner, Neelakanteswar Aluru, Diana G. Franks, Jared V. Goldstone, Bryan W. Clark, Diane E. Nacci, Lisa Truong, Jane La Du, Robyn L. Tanguay

Humans are exposed to chemicals such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), polychlorinated biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs) that cause toxicity through activation of the aryl hydrocarbon receptor (AHR). There is inter-individual variation in sensitivity to effects of AHR ligands, but it is not fully explained by variation in the human AHR, suggesting that other components of the AHR pathway are involved in controlling sensitivity. A clue to the genetic mechanisms underlying differential sensitivity to AHR agonists has emerged recently from studies of fish populations with multi-generational exposure to PCBs, TCDD, and PAHs. Four populations of the Atlantic killifish (*Fundulus heteroclitus*) have independently evolved resistance to these toxicants, resulting in a >100-fold reduction in sensitivity. Genomic studies of these populations identified the AHR chaperone AHR-interacting protein (AIP/Ara9/XAP2) as the strongest candidate resistance gene. However, the precise role of AIP in the mechanism of resistance is unknown. In addition to its role in the AHR complex, AIP is a susceptibility gene for human Familial Isolated Pituitary Adenoma (FIPA); dozens of SNPs in AIP predispose to this disease. To understand the role of AIP in the toxicity of dioxin-like compounds and how variation in AIP may affect chemical sensitivity, we are using killifish and zebrafish (*Danio rerio*) as models. We first used CRISPR-Cas9 to generate AIP loss-of-function alleles in killifish and zebrafish and are using them to examine the role of AIP in AHR function in vivo. We also will be using knock-in approaches to examine how AIP variants influence chemical sensitivity. Understanding the role of AIP in evolved resistance to AHR agonists may shed light on the factors that determine differential susceptibility of humans to dioxin-like compounds and on the role of AIP in human disease. [P42ES007381, P42ES016465, P30ES030287, R01ES032323, R01ES033888]

## Germplasm Cryopreservation & Open Hardware

### P22 Preserving amphibian biomedical models: developing of a repository for *Xenopus laevis* and *Ambystoma mexicanum*

**Lucia Arregui** (Louisiana State University)
Yue Liu, Jack Koch, M. Teresa Gutierrez-Wing, Terrence Tiersch

Amphibian biomedical models provide valuable resources for many branches of human health research. Production of transgenic and mutant lines has led to an expanding need for a cost-effective and efficient way to maintain these lines. Storage of cryopreserved germplasm in repositories can protect such lines and reduce the number of live animals maintained at the stock centers. In collaboration with the National Xenopus Resource (NXR) and the Ambystoma Genetic Stock Center (AGSC), we are developing a high-throughput cryopreservation pathway for *Xenopus laevis* and *Ambystoma mexicanum*. Currently, the NXR cryopreserves sperm while the AGSC does not. The feasibility of cryopreservation of sperm in *X. laevis* and spermatophores in A. mexicanum has been investigated previously. For *X. laevis*, a high variability among males in fertilizing capacity with frozen sperm have been found with 30% of them failing to produce enough embryos to recover a line. For A. mexicanum post-thaw sperm quality evaluation and use through in vitro fertilization have not been standardized. We tested the effect of the cryoprotectant (DMFA, DMSO, methanol, glycerol and ethylene glycol), its concentration (5%, 10% and 20%), the addition of sugar (sucrose or trehalose) and the freezing rate (2, 5, 10, 20, 40 and 60ÂºC/min) on sperm quality. For *X. laevis* the fertilizing capacity of testicular sperm frozen at different sperm concentrations (30, 50 and 100 x 106 cells/mL) was assessed. For A. mexicanum we analyzed the effect of sperm concentration 4Ã—105 to 106 and 10Ã—106 sperm/mL) and oocyte storage (5, 10 and 20 min in water or Holtfreter's solution at 120 mOsmol/kg) in the fertilization capacity of fresh sperm obtained after hormonal induction and abdominal massage. These results will allow reducing post-thaw variability among *X. laevis* males, setting standards for samples to be accepted into the repository at the NXR and establishing a repository at AGSC.

### P24 Developing an accessible and scalable cryopreservation pathway for the biomedical model California sea hare, *Aplysia californica*, with generalizability to other marine invertebrates

**Jack Koch** (Louisiana State University)
Allyssa M. Oune, Owen Plaisance, Marwan Okeil, Sarah Bodenstein, Lucía Arregui, Yue Liu, M. Teresa Gutierrez-Wing, and Terrence Tiersch

The safeguarding of economically important agricultural species has been driven by storing, evaluating, and distributing genetic resources as cryopreserved germplasm maintained in repositories. The shift to develop cryopreservation technologies has been slow within the broader scientific community, especially for aquatic biomedical model systems. A cryopreservation pathway that is generalizable to a variety of organisms across biological levels of organization can provide a foundation for repository development and a means for addressing cross-taxa challenges. In collaboration with the National Resource for Aplysia, we are developing a generalizable cryopreservation pathway, including quality management and economics, that can be applied to the California sea hare, *Aplysia californica*, with the intention of extending the pathway approaches to other aquatic invertebrates such as imperiled corals. This sea hare is a biomedical model gastropod used to examine neural

development, behavior, and aging. Inbreeding is detrimental to early life stage development in this and many other species and must be accounted for during pairings and when wild broodstock animals are difficult to obtain. Repository storage of frozen material will provide opportunities for the research community to create and maintain mutant, transgenic, and gene-edited lines and to preserve genetic diversity which is advantageous for several reasons beyond inbreeding. Cryopreservation and repository development comes with many challenges, including multiple related to tissue types and developmental stages. For example, the sea hare presents the challenges of encapsulation of multiple embryos within capsules and semi-rigid strands and is therefore more like cryopreserving tissues rather than cells. By developing cryopreservation pathways with model organisms, we can leverage existing resources and information to bring much-needed generalization, scalability, and application to other aquatic species.

**P26 Simulation modeling of zebrafish cryopreservation development and operation at an aquatic biomedical repository**

**Sarah Bodenstein** (Louisiana State University)
Fidan Abdullayeva, Zoltan Varga, Terrence Tiersch

Aquatic biomedical model organisms are an essential part of advancing our understanding of human health. To that end, laboratories across the globe have produced thousands of mutant and transgenic lines to generate models for human diseases and to validate new drugs. With all this advancement, however, relatively little work has been dedicated towards developing reliable, high-throughput storage methods for these valuable genetic resources. With proper storage, such as in a germplasm repository, the number of live populations that must be maintained can be reduced and valuable lines can be protected from loss due to natural disasters, accidents, and disease. The Zebrafish International Resource Center (ZIRC) has invested time and resources in cryopreservation and repository development. Over the years ZIRC has developed a standardized cryopreservation pathway and stored thousands of genetic lines for the zebrafish research community. However, this success has not been replicated in other stock centers and the ZIRC process for building and maintaining a repository has not been formally studied. To encourage repository development for other biomedical models and to improve the ZIRC repository system, this study used discrete-event simulation modeling to analyze the cryopreservation pathway and make recommended improvements. Such models must reflect â€œreal-worldâ€ working conditions and are used to simulate key outputs, such as calculating production capacity over time (throughput) or identifying limiting steps in the process (bottlenecks). With these models, decisions can be made to eliminate wastes in current systems. In addition, models can simulate multiple alternative scenarios such as hiring more operators or expanding production capacity to improve quality or reduce wastes and costs. Simulation modeling is a powerful tool that can improve the repository system at ZIRC and facilitate repository development at other biomedical stock centers.

## Neural Circuits & Diseases

### P28 Zebrafish Fyn kinase models of microglia activation and inflammatory signaling associated neurodegeneration

**Sahiba Siddiqui** (Iowa State University)
Fang Liu, Anumantha Kanthasamy, Maura McGrail

Cellular kinases play a central role in protein aggregation in dementia and neurodegeneration and their involvement in inflammatory signaling in Alzheimer's Disease (AD) and related neurodegenerative diseases (ADRD) is now emerging. The Src family member Fyn kinase mediates AD amyloid ï□¢ and tau protein pathophysiology, and more recently has been shown to regulate the microglia PKC-ï□¤/NF-ï□«ï□¢ inflammatory signaling in response to ï□¡-synuclein aggregate uptake. While protein aggregation and microglia activation reinforce neuroinflammation, the cellular mechanism at the intersection of these pathways is incompletely understood. An in vivo cell type-specific model of Fyn signaling is needed to reveal new insights into neuroinflammation, and to provide an effective translational tool for chemical and genetic screens to identify novel Fyn effectors of neurodegeneration. To address this need, we built an in vivo zebrafish Gal4:UAS model of Fyn signaling that allows cell type specific expression of constitutively active Fyn. Neural specific FynY531F expression leads to behavioral and neurodegeneration phenotypes that recapitulate zebrafish PD locus knockdown and neurotoxin models. Dopaminergic neuron loss in the 3-5 day old larval brain diencephalon correlates with an increase in brain microglia and expression of inflammatory cytokines Il-1β, IL-12β and TNF-α. Src, PKC-□/NF-□□, and caspase-1 chemical inhibitors suppress dopaminergic neuron loss, demonstrating neurodegeneration and inflammatory signaling in response to neuronal Fyn activity are dependent on activation of these pathways. We recently recovered a novel microglia-specific mpeg1.1-KalTA4 driver, using GeneWeld CRISPR targeted integration, that will allow us to investigate the cell autonomous role of Fyn in microglia activation. Together our zebrafish cell type-specific models of Fyn neurodegeneration provide novel in vivo platforms for proteomic and genetic screens to profile the Fyn neuroinflammation network.

### P30 Sleep deprivation induces changes to dopaminergic cell populations in larval zebrafish

**Beatrice Tynan** (William & Mary)
Belle Buzzi, Emily Yu, Jennifer Bestman

Parkinson's disease (PD) is one of the most prevalent and debilitating neurodegenerative diseases. It is characterized by the loss of dopamine-producing neurons in the substantia nigra. Half of individuals suffering from REM Sleep Behavior Disorder will develop Parkinson's within ten years, but it is unknown whether sleep disruption has a causal role or is an early symptom of the disease. We are developing a zebrafish model to characterize the molecular mechanisms that tie these conditions together, as zebrafish are known to be excellent models of both neurodegeneration and sleep. Since zebrafish are maintained on a 14:10 light/dark cycle, we used light exposure to induce sleep deprivation in 7dpf larvae and investigated changes to dopaminergic cell populations in the ventral diencephalon, the area of the zebrafish brain homologous to the human substantia nigra. We labeled the dopaminergic populations in fixed larvae using an antibody against tyrosine hydroxylase, a protein involved in the biosynthesis of dopamine, in combination with cell death markers. Considered scavengers of cellular debris, aggregating microglia indicate cell death. By

microinjecting IB4-Isolectin, an in vivo microglia marker, into the brain ventricle we will determine whether they aggregate in the ventral diencephalon. Whole-brain 3D image stacks were taken using a confocal microscope, and we have been developing open-access image analysis tools to characterize dopaminergic cell populations through both manual coded counting and automated analysis. Our data suggest three nights of consecutive sleep deprivation cause significant changes to dopaminergic cell populations in zebrafish larvae.

## Aquatic Skeletons & Application to Human Disorders

### P32 Molecular control of chondrocyte hypertrophy: an evolutionary approach

**Michael Palmer** (Marine Biological Laboratory)
Andrew Gillis

In mammals, cartilage is predominantly an embryonic tissue: the vast majority of cartilage is replaced by bone during the processes of hypertrophy and ossification. Cartilage persists in relatively few places within the adult skeleton (e.g. in joints, as articular cartilage) and has very limited capacity for spontaneous repair, which can lead to debilitating joint pathologies like osteoarthritis after injury. Stem cell-based approaches for cartilage injury treatment are currently hindered by the relative instability of mammalian cartilage cells (chondrocytes) in vitro and upon implantation into an injury site. Chondrichthyan fishes (sharks, skates, and rays), on the other hand, have undergone an evolutionary loss of bone, instead possessing a skeleton that is composed almost entirely of pre-hypertrophic cartilage throughout life. We are studying cartilage development in the skate (Leucoraja erinacea) to discover gene expression correlates of this permanent cartilaginous skeleton. We have used single-cell RNA-sequencing to identify genes that are differentially expressed both between differentiating and mature skate chondrocytes, and skate and mammalian skeletal cell types. Combining this approach with spatial validation using multiplexed in situ hybridization by chain reaction, we have found that skate post-embryonic chondrocytes retain expression of genes encoding inhibitors of the Wnt signaling pathway. This stands in contrast to mammals, where genes encoding orthologous Wnt inhibitors are downregulated with hypertrophy and ossification. These observations are consistent with the role of Wnt signaling in the differentiation/maturation of chondrocytes and suggest that modulation of Wnt signaling may have contributed to the evolution of a permanent pre-hypertrophic cartilaginous skeleton in chondrichthyans. This also points to manipulation of Wnt signaling as a promising avenue for further investigation in cell-based therapies for human cartilage diseases.

## Genome Engineering in Aquatic Models

### P34 *adgre5*: New *Xiphophorus* melanoma tumor suppressor gene

**Mateo Garcia** (Xiphophorus Genetic Stock Center, Texas State University)
Molly Schumer, Mateus Adolfi, Svenja Meierjohann, Gil Rosenthal, Manfred Schartl

Hybridization provides a great resource to pinpoint genes of interest, explore adaptative processes and molecular mechanisms underlying adaptation, and identify new disease-causing and disease-regulating genes. New mutations arising in diverging species can interact negatively in hybrids, generating what is known as hybrid incompatibilities. A famous example of hybrid incompatibility comes from laboratory hybrids between distantly related *Xiphophorus* species that develop malignant melanoma. This study utilizes an ongoing

hybridization process in nature in two other *Xiphophorus* species to study the genetic basis and evolutionary persistence of melanoma in the wild. *Xiphophorus birchmanni* is polymorphic for a coloration pattern on its caudal fin called spotted caudal (Sc). *X. malinche* lacks this pattern. *X. birchmanni* – X*. malinche* hybrids are also polymorphic regarding Sc and its expression can vary from a few black spots to extremely malignant melanoma. To identify the genetic basis of the trait, we performed a Genome Wide Association Study in a *X. birchmanni* population and determined that a previously known potent oncogene (*xmrk*) is responsible for driving the expression of the pattern. This was followed by a population ancestry and admixture mapping study that proposed *adgre5* and *xmrk* as the genes responsible for the hybrid incompatibility causing melanoma. We performed functional cell culture and transgenic experiments to determine that *X. birchmanni* 's *adgre5* allele acts as a tumor suppressor gene. To our knowledge, this is the only study that combines genomics and molecular biology techniques in an integrative approach to identify and functionally test a hybrid incompatibility (melanoma) to the single gene level in naturally occurring hybridizing species.

## Open Topic

### P36 Platelet Activating Factor augments FcR expression in sea urchin spermatozoa

**Nicole Russell** (University of South Carolina School of Medicine Greenville)
Sarah Feingold, Sanjana Mandilwar, Renee Chosed, William E. Roudebush

Introduction: Platelet-activating factor (PAF) is a signaling phospholipid that has a variety of reproductive roles, e.g., sperm capacitation and the acrosome reaction. PAF's mechanism action involves G-protein receptor activation induction of intracellular calcium, which enhances sperm motility. In nonreproductive cells, the Fc receptor (FcR) functions in ligand-triggered transmission signals across the plasma membrane which results in alteration in secretion, exocytosis, and increased intracellular calcium. This study looked at the impact of PAF on FcR expression in sea urchin spermatozoa.
Methods: Sea urchin sperm were exposed to exogenous PAF (10-7M; 15 minutes) in sea water. Samples were processed (over time) via a commercially available FcR assay (Arex Life Sciences) and analyzed via flow cytometry.
Results: A significant (P=0.01) difference (64.30%) in FcR wave activity between the PAF (mean 3.286) and control (2.0) groups was observed. Within waves, there were more (83.31%) high points (crests) in the PAF (mean 1.571) group than the control (mean 0.857) group. Similarly, there were more (49.96%) low (troughs) points in the PAF (mean 1.714) group than the control (mean 1.143) group. Additionally, PAF elongated the fertility window over untreated specimens.
Discussion: These preliminary results show a relative positive change in FcR expression when spermatozoa are exposed to PAF. The FcR functions in ligand-triggered transmission of PAF via a G-protein coupled receptor mediated pathway leading to alterations in secretion, exocytosis, and cellular metabolism. Additionally, in human spermatozoa, the FcR on sperm cells has been shown to follow a cyclic pattern of expression over time that impacts sperm functionality and fertilization potential. Provided the known mechanism of action of PAF on spermatozoa physiology, the data suggests that the interaction of PAF and FcR play a pivotal role in sperm activity and fertilization potential.

**P38** **Observations of platelet activating factor production by sea urchin embryos – The transverse wave hypothesis**

**Nicole Russell** (University of South Carolina School of Medicine Greenville)
Sarah Feingold, Sanjana Mandilwar, Renee Chosed, William E. Roudebush,

Introduction: Many endogenous elements impact human embryo development. One such element is platelet-activating factor (PAF, a signaling phospholipid) and is required for preimplantation growth. The extent by which PAF improves embryo development is not fully known. In other animal models, PAF increases intracellular calcium which facilitates embryo growth, cell division and gene expression. There is limited information on PAF production by the sea urchin embryo. This study investigated temporal PAF production by sea urchin embryos.
Materials and Method: Sea urchin (Lytechinus variegatus) oocytes were inseminated with sperm and cultured, two-cell embryo stage (~70 minutes). Two-cell embryos were cultured (50 embryos/replicate; 3 replicates/collection point) in filtered sea water (50ï□-L) under washed mineral oil for up to 24 hours. Over the 24-hour period, embryonic culture drops were collected (8 different points) and stored at -20oC. Embryo PAF levels were assessed via a commercially available ELISA kit (Abcam, Boston, MA) per manufacturer's instructions.
Results: Embryo PAF (ePAF) levels were highest at 2- (0.64 ng/mL) and 9-hours (0.68 ng/mL) and lowest at 3- (0.52) and 24-hours (0.53). Production of ePAF appears as a transverse wave and suggests that ePAF undergoes alternating, perpendicular movements about the mean (0.598 ng/mL) and moves in a direction perpendicular to the direction of embryo development (wave propagation) through the cell stages.
Discussion: These results confirm that PAF is produced by the sea urchin embryo and the first to show temporal production. Additionally, ePAF by the sea urchin embryo appears in a series of waves (crests/troughs) depicting a transverse wave which may direct cleavage. Provided the known PAF mechanism of action on embryo physiology, the data suggests a positive interaction between it and early cleavage events. Additional studies are required to further elucidate how ePAF propagates cell division in the early embryo.

# List of Participants

| First Name | Last name | Email | Affiliation |
|---|---|---|---|
| Michael | Addo | mnaddo@uab.edu | University of Alabama |
| Carrie | Albertin | calbertin@mbl.edu | Marine Biological Laboratory |
| Shahid | Ali | shahidali@uchicago.edu | The University of Chicago |
| Chris | Amemiya | camemiya@ucmerced.edu | Univ. California, Merced |
| Khai | Ang | khaichung@gmail.com | Penn State College of Medicine |
| Chiara | Anselmi | chiara90@stanford.edu | Stanford University |
| Lucia | Arregui | larregui@agcenter.lsu.edu | AGGRC (LSU AGCenter) |
| Anne | Baldino | axb1998@miami.edu | University of Miami |
| Elizabeth | Bearce | bearceea@uoregon.edu | University of Oregon |
| Jennifer | Bestman | jebestman@wm.edu | William & Mary, Williamsburg |
| Peggy | Biga | pegbiga@uab.edu | University of Alabama |
| Sarah | Bodenstein | sboden2@lsu.edu | AGGRC LSU AgCenter |
| Ingo | Braasch | braasch@msu.edu | Michigan State University |
| Shawn | Burgess | burgess@mail.nih.gov | National Human Genome Research Institute |
| Sarah | Burton | sburton@mbl.edu | Marine Biological Laboratory |
| Kate | Castellano | kate.castellano@gmgi.org | Gloucester Marine Genomics Institute |
| Miguel | Contreras | Miguel.Contreras@nih.gov | National Institute of Health |
| Kelsey | Coppenrath | kcoppenrath@mbl.edu | Marine Biological Laboratory |
| Jacob | Daane | jdaane@uh.edu | University of Houston |
| Mark | Daly | MDaly@cantatabio.com | Cantata Bio |
| Elizabeth | DiBona | edibona@islander.tamucc.edu | Texas A&M University-Corpus Christi |
| Stephen | Douglass | sdouglass@concoll.edu | Connecticut College |
| Valeria | Dountcheva | v.dountcheva001@umb.edu | University of Massachusetts Boston |
| Greg | Driscoll | gdriscoll@cortekssystems.com | Cortecks Systems |
| Kang | Du | dukang1117@outlook.com | Texas State University |
| Erik | Duboue | eduboue@fau.edu | Florida Atlantic University |
| Timothy | Duerr | duerr.t@northeastern.edu | Northeastern University |
| David | Duffy | duffy@whitney.ufl.edu | University of Florida |
| Jeffrey | Farrell | jeffrey.farrell@nih.gov | NICHD |
| Lynne | Fieber | lfieber@miami.edu | University of Miami |
| Khalid | Freij | kfreij95@uab.edu | University of Alabama |
| Brigitte | Galliot | brigitte.galliot@unige.ch | University of Geneva |
| Mateo | Garcia | mateog90@gmail.com | Texas State University |
| Andrew | Gillis | agillis@mbl.edu | Marine Biological Laboratory |
| Gary | Gorbsky | GJG@omrf.org | Oklahoma Medical Research Foundation |
| Ryan | Gray | ryan.gray@austin.utexas.edu | University of Texas at Austin |
| Kristin | Gribble | kgribble@mbl.edu | Marine Biological Laboratory |
| Maria Teresa | Gutierrez-Wing | mwing@agcenter.lsu.edu | Louisiana State University Ag Center |
| Mark | Hahn | mhahn@whoi.edu | Woods Hole Oceanographic Institution |
| Amro | Hamdoun | hamdoun@ucsd.edu | University of California San Diego |

| | | | |
|---|---|---|---|
| Chrissy | Hammond | chrissy.hammond@bristol.ac.uk | University of Bristol, United Kingdom |
| Matthew | Harris | Harris@genetics.med.harvard.edu | Harvard Medical School |
| Noam | Hendin | noam.hendin@gmail.com | Tel Aviv University |
| Jonathan | Henry | jhenry@mbl.edu | Marine Biological Laboratory |
| Rachael | Heuer | rheuer@miami.edu | University of Miami |
| Veronica | Hinman | vhinman@andrew.cmu.edu | Carnegie Mellon University |
| Andrew | Hoadley | aphoadle@central.uh.edu | University of Houston |
| Mandë | Holford | mholford@hunter.cuny.edu | Hunter College/AMNH |
| Marko | Horb | Mhorb@mbl.edu | Marine Biological Laboratory |
| Luciana | Isaja | lucianaisaja@gmail.com | Universidad de Buenos Aires |
| Harini | Iyer | iyerh@stanford.edu | Stanford University |
| Erich | Jarvis | ejarvis@rockefeller.edu | The Rockefeller University<br>Howard Hughes Medical Institute |
| Rijith | Jayarajan | rjayarajan@islander.tamucc.edu | Texas A&M University - Corpus Christi |
| Scott | Juntti | sjuntti@umd.edu | University of Maryland |
| Paul | Katz | pkatz@umass.edu | University of Massachusetts Amherst |
| Michael | Kent | michael.kent@oregonstate.edu | Oregon State University |
| Maggie | Kettelberger | mkettelberger@wm.edu | William and Mary |
| Bill | Kilgore | bill.kilgore@aquaneering.com | Aquaneering |
| Hyunjin | Kim | kim2377@purdue.edu | Purdue University |
| Jack | Koch | jkoch@agcenter.lsu.edu | Louisiana State University AgCenter |
| Sovannarith | Korm | skorm@mbl.edu | Marine Biological Laboratory |
| Johanna | Kowalko | jok421@lehigh.edu | Lehigh University |
| Arjun | Krishnan | arjun.krishnan@cuanschutz.edu | University of Colorado Anschutz Medical Campus |
| Nicholas | Kron | n.kron@umiami.edu | University of Miami |
| Ankur | Kumar | ankurk@iastate.edu | Iowa State University |
| Ramon | Lavado | Ramon_Lavado@baylor.edu | Baylor University |
| Alyssa | Liguori | aliguori@mbl.edu | MARINE BIOLOGICAL LABORATORY |
| Yue | Liu | yliu@agcenter.lsu.edu | Louisiana State University Agricultural Center |
| Ben | Lovely | ben.lovely@louisville.edu | University of Louisville |
| Yuan | Lu | y_l54@txstate.edu | Texas State University |
| Deirdre | Lyons | d1lyons@ucsd.edu | University of California, San Diego |
| Mirjana | Malnar Črnigoj | mirjana.malnar@pmu.ac.at | Paracelsus Medical University, Salzburg |
| Tom | Marcone | tmarcone@iwakiamerica.com | Iwaki America |
| Sian | Martin | sian.martin@port.ac.uk | European Xenopus Resource Centre |
| Sabateeshan | Mathavarajah | smathavarajah@dal.ca | Dalhousie University |
| Catherine | McCusker | catherine.mccusker@umb.edu | University of Massachusetts Boston |
| Sean | McNamara | smcnamara@iwakiaquatic.com | Iwaki America |
| Eric | Moore | emoore@iwakiaquatic.com | Iwaki Aquatic Systems |
| Jennifer | Morgan | jmorgan@mbl.edu | Marine Biological Laboratory |
| Christian | Mosimann | christian.mosimann@cuanschutz.edu | University of Colorado School of Medicine |
| Jacob | Musser | jmmusser@gmail.com<br>jacob.musser@yale.edu | European Molecular Biology Laboratory,<br>Yale University |

| | | | |
|---|---|---|---|
| Sam | Norris | sammynorris@gmail.com | University of California |
| Michael | Palmer | mpalmer@mbl.edu | Marine Biological Laboratory |
| Ann | Petersen | ann.petersen@noaa.gov | Northeast Fisheries Science Center |
| Annapurna | Poduri | annapurna.poduri@childrens.harvard.edu | Harvard Medical School |
| Ken | Poss | kenneth.poss@duke.edu | Duke University |
| John | Postlethwait | jpostle@uoregon.edu | University of Oregon |
| Rock | Pulak | rpulak@unionbio.com | Union Biometrica |
| Eric | Randolph | ecr77@miami.edu | University of Miami |
| Sven | Reischauer | S.Reischauer@kerckhoff-fgi.de | Justus-Liebig University, Giessen, Germany |
| Jacques | Robert | jacques_robert@urmc.rochester.edu | University of Rochester Medical Center |
| Javier | Rodriguez-Casariego | javirodr@fiu.edu | University of Puerto Rico,<br>University of Miami,<br>Florida International University |
| Crystal | Rogers | crdrogers@ucdavis.edu | University of California, Davis |
| Nicolas | Rohner | nro@stowers.org | Stowers Institute |
| William | Roudebush | roudebus@greenvillemed.sc.edu | University of South Carolina |
| Zubaida | Saifudeen | zubaida.saifudeen@nih.gov | National Institute of Health |
| Rachelle | Saint-Fort | ras778@psu.edu | Penn State College of Medicine |
| Michael | Sandel | msandel@uwa.edu | University of West Alabama |
| Elizabeth | Schabot | exs1207@miami.edu | University of Miami, Miami, FL |
| Manfred | Schartl | phch1@biozentrum.uni-wuerzburg.de | Texas State University |
| Michael | Schmale | mschmale@rsmas.miami.edu | University of Miami |
| Igor | Schneider | igors@lsu.edu | Louisiana State University |
| Patricia | Scneider | pschneider@lsu.edu | Louisiana State University |
| Frauke | Seemann | frauke.seemann@tamucc.edu | Texas A&M University-Corpus Christi |
| Thomas | Sharpton | Thomas.sharpton@oregonstate.edu | Oregon State University |
| Sahiba | Siddiqui | sahiba@iastate.edu | Iowa State University |
| Christopher | Smaga | christopher.smaga@uga.edu | University of Georgia,<br>Savannah River Ecology Lab |
| Jeramiah | Smith | jjsmit3@uky.edu | University of Kentucky |
| Erika | Soria | iyx4@txstate.edu | Texas State University |
| Tyler | Square | square@berkeley.edu | University of California, Berkeley |
| Lance | Squires | lsquires@tecniplastusa.com | Tecniplast USA |
| Alberto | Stolfi | alberto.stolfi@biosci.gatech.edu | Georgia Institute of Technology |
| Savini | Thrikawala | sthrika@clemson.edu | Clemson University |
| Terrence | Tiersch | ttiersch@agcenter.lsu.edu | Louisiana State University AgCenter |
| Stephen | Treaster | stephen_treaster@hms.harvard.edu | Harvard Medical School,<br>Boston Children's Hospital |
| Beatrice | Tynan | batynan@wm.edu | William and Mary |
| Zoltan M. | Varga | zoltan@zebrafish.org | University of Oregon, ZIRC |
| Gert Jan | Veenstra | g.veenstra@science.ru.nl | Radboud University |
| Brittney | Voigt | bpvoigt@utexas.edu | The University of Texas at Austin |
| Randal | Voss | srvoss@uky.edu | University of Kentucky |
| Joseph | Walewski | christopher.smaga@uga.edu | Connecticut College |

| | | | |
|---|---|---|---|
| Wesley | Warren | warrenwc@missouri.edu | University of Missouri |
| Kristine | Willett | kwillett@olemiss.edu | University of Mississippi |
| Christoph | Winkler | dbswcw@nus.edu.sg | National University of Singapore |
| Lihua | Ye | lihua.ye@osumc.edu | The Ohio State University |
| Masato | Yoshizawa | yoshizaw@hawaii.edu | University of Hawaii at Manoa |
| GuangJun | Zhang | gjzhang@purdue.edu | Purdue University |
| Fanghemei | Zhang | fanghemei.zhang001@umb.edu | The University of Massachusetts Boston |
| Chongbei | Zhao | drs@stowers.org | Stowers Institute |