EUROPEAN ZEBRAFISH HUSBANDRY ASSOCIATION MEETING

PROGRAM AND BOOK OF ABSTRACTS

16-18 October, 2024

Gödöllő, Hungary

HUNGARIAN UNIVERSITY OF AGRICULTURE AND LIFE SCIENCES

Pushing the Limits: Using VIE to Identify Small Fish

Most tags just don't fit in small-bodied and early life stages of fish, but we still need to identify them, preferably without biasing our data. The options are further limited when many batches or individual identification is required. Visible Implant Elastomer™ (VIE) is injected internally but remains externally visible. The size of a tag is controlled by the tagger, so it is easily adapted to very small fish.

VIE has proven to be very useful for tagging zebrafish. Anita Rácz has developed a detailed protocol for zebrafish that emphasizes animal welfare. Anita will be presenting a hands-on tagging workshop—join her to learn and try the technique and to explore how it might apply to your work.

If your research includes tagging larval zebrafish, then recent work by Frederickson will complement Anita's protocol. His group has developed a method for tagging larval zebrafish as early as 2 days post-fertilization.

VIE is used world wide for a wide variety of animals including hundreds of species of fish, crustaceans, amphibians, cephalopods, reptiles, insects, and small mammals. Please contact us if we can help with your project.


Photo: Anita Rácz

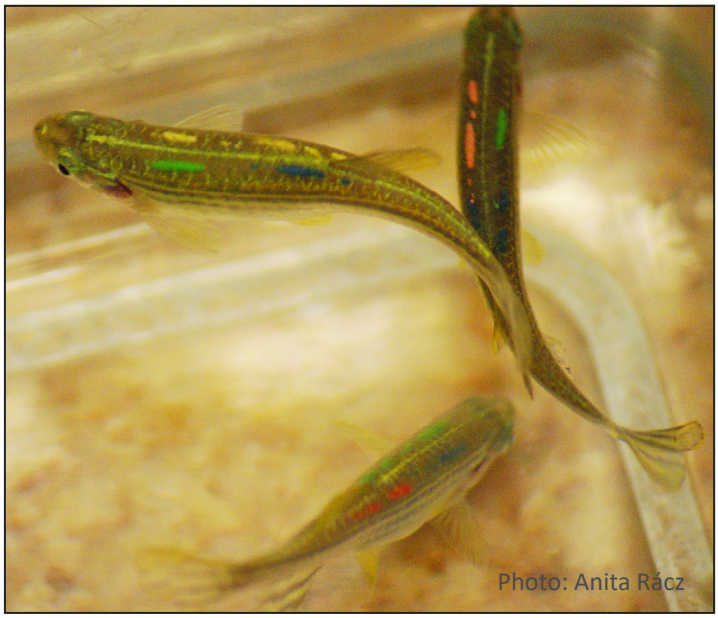
Photo: Anita Rácz

Photos: VIE is injected under the skin of zebrafish with a syringe. Colors and tag locations can be combined to create a coding scheme as shown. VIE is available in 10 colors (left). Six fluoresce under a VI Light for improved visibility and tag detection (right).

Rácz, A., B. Allan, T. Dwyer, D. Thambithurai, A. Crespel, S.S. Killen. Identification of Individual Zebrafish (*Danio rerio*): A Refined Protocol for VIE Tagging Whilst Considering Animal Welfare and the Principles of the 3Rs. Animals **2021**, 11, 616. https://doi.org/ 10.3390/ani11030616

Frederickson, S., B. Carrington, T. Clark 2023. Zebrafish Injectable Plastic for Identification Tagging (ZIP IT) for larvae to adults using a fluorescent Visible Implant Elastomer. Methods X, Volume 11, 102340 https://methods-x.com/article/S2215-0161(23)00337-0/fulltext

Northwest Marine Technology, Inc.

www.nmt.us Anacortes, Washington, USA

TABLE OF CONTENTS

ORGANIZING AND SCIENTIFIC COMMITTEE

Anita Rácz
Zebrafish Facility Manager at Hungarian University of Agriculture and Life Sciences (MATE) Szent Istvan Campus, Gödöllő,
Zebrafish Facility Manager at Eötvös Lorand University (ELTE), Budapest, Hungary

Claire Allen, PhD
Biological Services Aquarium (BSA) Manager Faculty of Science, University of Sheffield, UK
ZHA Executive of Social Media and Outreach

Mollie Millington
BRF Unit Manager & Senior Research Scientist at the Francis Crick Institute London, UK

Joana Monteiro, PhD
Manager of Fish Platform at Champalimaud Foundation, Portugal

Ana Cristina Borges
Head of the Aquatic Platform at GIMM – Gulbenkian Institute for Molecular Medicine, Portugal & Vice-President of the ZHA.

Zsolt Csenki-Bakos, PhD
Deputy head of Environmental Toxicology department,
Senior research fellow at Hungarian University of Agriculture and Life Sciences (MATE)- Institute for Aquaculture and Environmental Safety, Szent Istvan Campus, Gödöllő, Hungary

István Szabó, PhD
Head of Environmental Toxicology department,
Associate professor at Hungarian University of Agriculture and Life Sciences (MATE)- Institute for Aquaculture and Environmental Safety, Szent Istvan Campus, Gödöllő, Hungary

László Orbán, PhD
Senior Research Advisor and Professor (adjunct) Frontline Fish Genomics Research Group, Department of Applied Fish Biology, Hungarian University of Agriculture and Life Sciences (MATE), Institute of Aquaculture and Environmental Safety, Georgikon Campus, Keszthely, Hungary

GENERAL INFORMATION

Venue of the Meeting
Agricultural Machinery and Technology Curiosity Museum, Gödöllő, Hungary
2100 Gödöllő, Páter Károly út 1

Date: 16-18 October, 2024

Onsite Registration

The registration desk will be open from 12 pm on 16th October, 2024. Location: Exhibition Hall

Name badge:

Please wear your name badges at all times during the conference.

Parking
Free parking is available next to the museum building.

Changing money
Hungary has its own local currency, the Hungarian Forint. You can easily get cash directly through an ATM machine. There is an ATM machine at Hungarian University of Agriculture and Life Sciences (MATE) as well. All major bank cards are also widely accepted.

Networking Opportunities

Wednesday, 16th October 2024, 6:30 pm, exhibition hall: **Welcome Reception and poster session**
Thursday, 17th October 2024, 7:00 pm, Lázár Equestrian Park: **Gala dinner**

Gala dinner

Bus departure at 6:30 pm from the conference venue. Do not forget to bring your ticket with you.

Gala dinner tickets in limited number are available at the registration desk. Dress code: business casual.

Exhibition, Trade show

Please visit the booths of the vendors. Bring business cards or have a digital way to share your contact information. Take part in the quiz game to win one of the valuable gifts.

Drawing during the closing session. Lunches and coffee breaks will be served at the exhibition hall.

Accommodation: For details of the official conference hotels and their proximity to the venue please visit the website of the conference. Breakfast is available at all of them, the price is included in the accommodation fee. No transport will be provided between the hotels and the meeting venue.

Insurance

Altagra, the official PCO of EZHAM 2024 carries a liability insurance for its activities. Liability insurance does NOT cover any personal injuries to participants resulting from an accident, travel damage, flight delays and baggage damage.

Video recording

Please be informed that video recording will take place during the conference. The recordings will be used for archival purposes, future reference and promotional materials. By attending, you consent to being recorded and to the use of the recordings as described.

Zebrafish Husbandry Association

Join at https://zhaonline.org/

The global community that cares about all things #zebrafish

@ZHAOnline

@zebrafishhusbandry

@zebrafishhusbandryassociation

Zebrafish Husbandry Association

WELCOME BY ZHA

On behalf of the Zebrafish Husbandry Association (ZHA), we are honored to welcome you to the European ZHA Meeting 2024. This gathering represents a unique opportunity for us to come together as a community dedicated to the advancement of zebrafish husbandry and research. As an organization committed to promoting best practices, education, and innovation in zebrafish care, we are excited to engage with experts, share our collective knowledge, and explore the latest advancements in the field. We believe that through collaboration and the exchange of ideas, we can continue to enhance the standards of zebrafish husbandry, ultimately contributing to more robust and reliable research outcomes. We look forward to inspiring discussions, new collaborations, and a memorable conference experience. Thank you for being part of this vital community, and we wish you an enriching and successful event.

The ZHA Executive Board

IDEXX **BioAnalytics**

Aquatic Health Monitoring & Diagnostics

UNPARALLELED EXPERTISE AND SUPPORT IN AQUATIC DIAGNOSTICS

Innovative testing solutions for aquatic models

+ Pathogen detection using real-time PCR
+ Environmental diagnostics available
+ Large portfolio of assays, including Zebrafish picornavirus (ZfPV1)
+ Support in health monitoring program design and data interpretation
+ Full-service microbiology laboratory with identification by MALDI-TOF mass spectrometry
+ Fast turnaround time

idexxbioanalytics.eu

idexxbioanalytics-europe@idexx.com

WELCOME BY MATE

Dear Reader,

Our old dream is coming true, that in the fall of 2024, the Institute of Aquaculture and Environmental Safety of the Hungarian University of Agriculture and Life Sciences (MATE), can host the meeting of the Zebrafish Husbandry Association (ZHA) in Europe. Some years ago, we started thinking together with the members of the association to organize the well known ZHA Meeting, which is so popular overseas, here in the old continent. In 2020, Covid-19 virus got in the way, and for a few years we could have only met, even with our closest colleagues, within a strictly regulated environments or just on-line. I'm sure you still remember, at that time we could hardly dream of personal meetings, especially with the international community. Today this period seems to be history, but it was very important to draw conclusions from it. If we would order to sustain the normal quality of life of the human race, than a supportive and non-virtual social network and personal relationships within it, are at least as important as other necessities to satisfy the basic needs of life, such as healthy drinking water.

We need to understand and analyze the effects of the pandemic in the same way as we do in the case of other harmful effects that threaten our common health. Every day, you contribute with your work to us understanding more about the environmental effects that risking people and their possible short- and long-term health consequences. With the highly appreciated help of zebrafish, it is not only possible to identify agents that threaten human health, but also the effects that damage quality of natural aquatic ecosystems and, ultimately, the fresh water that sustains everyone. This small fish, as a model animal, could make acute effects visible and even predicts long-term (chronic) processes through the results of the experiments. We cannot ignore the fact that protecting our own health, and protecting the environment, claims the sacrifice of many fishes. For this reason, and quite rightly, the regulations on the usage of experimental animals have become increasingly strict in recent years, worldwide. Thus it is important not to hurt these animals unnecessarily and to keep them in conditions that are as optimal as possible for them. This fish species make such a great effort to protect both human and environmental health, that they deserve highly trained professionals who deal with them knowing and taking care of the up-to-date ethical standards of animal husbandry and welfare at the highest possible level. This is our shared responsibility.

During our event, our guests hopefully can learn about husbandry, get mastered methods and - thanks to our honorable sponsors - apply the state-of-the-art equipments, which can contribute to develop the zebrafish husbandry practices. I hope that all guests of ours will be able to obtain valuable information for your further improvement, and we thank again for the cooperation of the ZHA in organizing the meeting!

Welcome to Gödöllő, Hungary! Minden jót kívánok!

Dr. Szabó István,

head of department

Dept. of Environmental Toxicology

zebrabase

www.zebrabase.org

About

Zebrabase is a web-based, scalable, and cross-platform zebrafish husbandry management database that can be adapted for any other species. It is built on three main cornerstones: comprehensive animal overview, interactive breeding history, and advanced management features.

Our aim is to provide animal facility solutions for research institutions. Zebrabase is suitable for all facilities that are looking to improve their husbandry workflow regardless of the size and layout.

Features

Comprehensive animal management

- Real-time facility management
- Complete logging of all animal procedures
- Instant access to the stock records via QR labels

Statistics and reporting

- Detailed statistics visualized in interactive charts
- Module for billing based on the number of occupied tanks
- Reporting for GMO and veterinary controls

Interactive facility view

- Tracking of animals in their positions, including their counts
- An intuitive and configurable web interface optimized for all devices

Animal reporting module

- Overview of used experimental animals
- Compliance with the EU Directive on the protection of animals used for scientific purposes
- Flexible selection of reporting parameters, including severity assessment

Detailed breeding history

- Visualization of crossing history
- Automatic genotype attribution of new stocks

Management features

- Calendar-based planning options and requests
- Built-in messaging system for user communication

Automatically generated QR labels

- Customizable template based on specific needs
- Compatibility with common label printers

Other features

- A complete history of all performed actions
- Customizable database parameters and user interface
- Multispecies module for managing multiple species

zebrabase

Institute of Molecular Genetics
of the Czech Academy of Sciences
Vídeňská 1083
142 20 Prague 4, Czech Republic

GENERAL ENQUIRIES
support@zebrabase.org
IMG

2nd European Zebrafish Husbandry Association Meeting

Final Program

Wednesday - 16th October, 2024.

12.00 – 14.00 Meeting Registration + facility tours + exhibition hall opening + sandwich lunch

12.30 – 13.00 facility tour – group 1 (max 15 people)
13.00 – 13.30 facility tour – group 2 (max 15 people)
13.30 – 14.00 facility tour – group 3 (max 15 people)

14.00 – 14.30 Welcome session by Organizing Committee, MATE, and ZHA

14.40 – 15.55 Opening session (part I)
Main Chair: Claire Allen **Co- Chair:** Anita Rácz

14.40 – 15.00 ENVIRONMENTAL ENRICHMENT
Lynne Sneddon
Department of Biological and Environmental Sciences, University of Gothenburg, Gothenburg, Sweden

15.00 – 15.05 Discussion

15.05 – 15.25 REDUCING THE ENVIRONMENTAL IMPACT OF ZEBRAFISH FACILITIES
J-P Mocho
DanioVet, Potters Bar, UK

15.25 – 15.30 Discussion

15.30 – 15.50 GENETICALLY ALTERED ZEBRAFISH AT THE CROSSROAD BETWEEN SCIENCE AND LAW
Livia D'Angelo[1,2]
[1]Dept Veterinary Medicine and Animal Production, University of Naples Federico II, Italy
[2]Federation of European Laboratory Animal Science Associations – FELASA

15.50 – 15.55 Discussion

15.55 – 16.30 Coffee break at exhibition hall

Wednesday

16.30 – 18.30 Opening session (part II)
Main Chair: Ana Borges **Co- Chair:** László Orbán

16.30 – 16.55 USING ENVIRONMENTAL DATA TO DRIVE IMPROVEMENTS IN WELFARE, MANAGEMENT, AND REPRODUCIBILITY
Christian Lawrence– remote
SmartLabs, Boston MA USA

16.55 – 17.00 Discussion

17.00 – 17.20 SEEKING TO BETTER ALIGN THE REGULATION OF ZEBRAFISH RESEARCH WITH WELFARE GOALS
Gregory Paull
University of Exeter, Geoffrey Pope Building, Stocker Road, Exeter, Devon, UK

17.20 – 17.25 Discussion

17.25 – 17.35 Presentation by IDEXX GmbH

17.35 – 17.50 Break

17.50 – 18.00 Presentation by Zebrabase – remote

18.00 – 18.05 Presentation by Aquaneering LLC

18.05 – 18.10 PHYSICAL AND SENSORIAL ENRICHMENT IN ZEBRAFISH AND MEDAKA: EVALUATION OF PREFERENCES, IMPACTS, AND WELFARE CONSIDERATIONS
Evgenia Dunaevskaya[1], Marco A. Vindas[1], Ian Mayer[1], Romain Fontaine[1], Anthony Peltier[1], Arturas Kavaliauskis[1], Eirill Ager Wick[1]
[1]The Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Ås, Norway.

18.10 – 18.15 PAIN IN ZEBRAFISH BEYOND FIN CLIPPING: THE CASE OF THE TRAUMATIC BRAIN INJURY MODEL
Ana Valentim[1], Beatriz Custódio[1,2], Elena van Os[1,3], Sofia Guimarães[1], Anna Olsson[1]
[1]i3S Institute for Research and Innovation in Health
University of Porto, Porto, Portugal
[2]Abel Salazar Biomedical Sciences Institute (ICBAS),
Universidade do Porto, Porto, Portugal
[3]School of Engineering and Applied Science,
Rotterdam University of Applied Sciences, Rotterdam, The Netherlands

Wednesday

18.15 – 18.20 ZEBRAFISH HEALTH MANAGEMENT - THE VETERINARY SIDE OF THE STORY

Márton Hoitsy[1,2], Krisztina Bali[3], János Gál[1], Árisz Ziszisz[1]
[1]Department of Exotic Animal and Wildlife Medicine, University of Veterinary Medicine, Budapest, Hungary
[2]Vet4Fish Kft., Szada, Hungary
[3]Department of Microbiology and Infectious Diseases, University of Veterinary Medicine, Budapest, Hungary

18.20 – 18.30 Discussion (collective discussion for the short talks)

18.30 – 20.30 Welcome reception and poster session at exhibition hall

Thursday - 17th October, 2024.

08.00 – Gate opening + Exhibition area + registration at site

08.30 – 10.30 EU Regulation Session
Main Chair: Mollie Millington **Co- Chair:** Zsolt Csenki-Bakos

08.30 – 09.45 IMPLEMENTING THE 3R UNDER THE AMENDED DIRECTIVE 2010/63/EU
Livia D'Angelo[1,2], J-P Mocho[2,3]
[1]Department Veterinary Medicine and Animal Production, University of Naples Federico II, Italy
[2]Federation of European Laboratory Animal Science Associations – FELASA
[3]DanioVet, Potters Bar, UK

09.45 – 09.55 Discussion

09.55 – 10.00 EFFECTS OF SPIRULIN-SUPPLEMENTED DIET ON ZEBRAFISH: A LONGITUDINAL STUDY
Ferdinando Flagiello[1], Antonio Palladino[2], Stefano Mazzoleni[2], Marcello Diano[3], Maria Raggio[1], Livia D'Angelo[1] Paolo De Girolamo[1]
[1]Dept. Of Veterinary Medicine and Animal Production, University of Naples Federico II, Naples, Italy;
[2]Dept. of Agricultural Sciences, University of Naples Federico II, Portici, Italy;
[3]M2M Engineering sas, Science Center, 80124 Naples, Italy.

10.00 – 10.10 ARE SHORT- AND MID-TERM FASTING IMPLICATED IN ZEBRAFISH WELFARE?
Maria Raggio[1], *Daniela Giaquinto[1], Chiara Attanasio[1], Antonio Palladino[2], Elena De Felice[3], Paolo de Girolamo[1], Livia D'Angelo[1]*
[1]Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Naples, Italy
[2]Department of Agricultural Sciences, University of Naples Federico II, Portici, Italy
[3]School of Bioscience and Veterinary Medicine, University of Camerino, Camerino, Italy

10.10 – 10.15 Presentation by Bionomus

10.15 – 10.30 Discussion (collective discussion for the short talks)

10.30 – 11.00 Coffee break at exhibition hall

Thursday

11.00 – 12.45 3Rs Session
Main Chair: László Orbán **Co- Chair:** Joana Monteiro

11.00 – 11.15 THE 3RS IN ZEBRAFISH RESEARCH
Kamar Ameen-Ali
School of Health and Life Sciences,
Teesside University, Middlesbrough, UK

11.15 – 11.20 Discussion

11.20 – 11.35 WELFARE ASSESSMENT, PAIN AND ANALGESIA
Lynne Sneddon
Department of Biological and Environmental Sciences,
University of Gothenburg, Gothenburg, Sweden

11.35 – 11.40 Discussion

11.40 – 11.55 HARMONISING RESEARCH FOR THE 3RS: TAILORING ZEBRAFISH HUSBANDRY FOR THEIR PURPOSED RESEARCH
Gregory Paull
University of Exeter, Geoffrey Pope Building, Stocker Road, Exeter, Devon, UK

11.55 – 12.00 Discussion

12.00 – 12.15 REDUCING SEVERE SUFFERING IN REGULATORY TOXICOLOGY TESTING USING FISHES
Chloe Stevens
Animals in Science Department,
RSPCA, Parkside, Chart Way, Horsham, UK

12.15 – 12.20 Discussion

12.20 – 12.35 SCORE SHEETS AND HUMANE ENDPOINTS
Linda Andersen
Stiftelsen Industrilaboratoriet (ILAB), Bergen, Norway

12.35 – 12.40 Discussion

12.40 – 12.45 Presentation by PLEXX B.V.

12.45 – 13.45 Lunch at exhibition hall

13.45 – 13.50 Presentation by RandW Associates (Armis) - remote

Thursday

13.50 – 14.35 Workshop introductory talks
Main Chair: István Szabó **Co- Chair:** Ana Borges

13.50 – 14.00 WORKSHOP1:

APPLYING 3RS TO GENOTYPING TECHNIQUES (EMBRYO, LARVAE)
Lisa Van Hateren
The University of Sheffield, UK

SKIN SWABBING: A REFINEMENT FOR DNA SAMPLING OF LABORATORY FISH
[1,2]Ceinwen Tilley
[1]Genetics and Genome Biology,
University of Leicester, Leicester, UK
[2]Neuroscience, Psychology and Behaviour,
University of Leicester, Leicester, UK

14.00 – 14.05 Discussion

14.05 – 14.15 WORKSHOP2:

VIE TAGGING WORKSHOP
[1,2]Anita Rácz
[1]Department of Genetics,
Eötvös Loránd University, Pázmány P.s. Budapest, Hungary
[2]Institute for Aquaculture and Environmental Safety,
Hungarian University of Agriculture and Life Sciences Szent István Campus,
Gödöllő, Hungary

14.15 – 14.20 Discussion

14.20 – 14.30 WORKSHOP3:

IMAGING AND BEHAVIOUR WORKSHOP
[1]Karishma Chhabria – remote, [2]Claire Allen
[1]Department of Physics,
University of California San Diego, USA
[2]Biological Services Aquarium (BSA) Faculty of Science,
University of Sheffield, UK

14.30 – 14.35 Discussion

14.35 – 15.00 Coffee break and posters at exhibition hall

Thursday

15.00 – 17.00 HANDS-ON WORKSHOPS
Main Chair: István Szabó **Co- Chair:** Ana Borges

15.00 – 15.40 APPLYING 3RS TO GENOTYPING TECHNIQUES (EMBRYO, LARVAE, SKIN SWAB)
15.40 – 16.20 VIE TAGGING WORKSHOP
16.20 – 17.00 IMAGING AND BEHAVIOUR WORKSHOP

18.30 Transfer to Gala dinner

19.00 – 22.00 Gala dinner – Lázár Equestrian Park
(Hungarian horse show + Traditional Hungarian dishes)

22.00 Transfer to the hotels

Friday - 18th October, 2024.

08.30 – Gate opening + Exhibition area

09.00 – 11.30 Husbandry Session
Main Chair: László Orbán **Co- Chair:** Mollie Millington

09.00 – 09.15 THE REPRODUCIBILITY CRISIS IN ZEBRAFISH RESEARCH – WHY NOT TELLING IS ONLY AN OPTION IN LAS VEGAS
Karin Finger-Baier
Department Genes – Circuits – Behavior, Max Planck Institute for Biological Intelligence, Martinsried, Germany

09.15 – 09.20 Discussion

09.20 – 09.30 ROLE OF WATER CHEMISTRY IN ZEBRAFISH WELFARE AND REPRODUCIBILITY OF RESEARCH STUDIES
Rodney Wilson – remote
University of Exeter, Exeter, UK

09.30 – 09.35 Discussion

09.35 – 09.45 DIETARY REQUIREMENTS FOR ZEBRAFISH
Joana Monteiro
Champalimaud Foundation, Lisbon, Portugal

09.45 – 09.50 Discussion

09.50 – 10.05 RUNNING A ZEBRAFISH CRYOPRESERVATION AND REDERIVATION SERVICE
Helen Horsler
The Francis Crick Institute, London, UK

10.05 – 10.10 Discussion

10.10 – 10.15 Presentation by ScienceTrack (Scientific Software Solutions)

10.15 – 10.20 Presentation by Tecniplast S.P.A.

10.20 – 10.50 Coffee break at exhibition hall

10.50 – 11.00 AGEING
Claire Allen
Biological Services Aquarium (BSA) Faculty of Science,
University of Sheffield, UK

11.00 – 11.05 Discussion

11.05 – 11.15 DEVELOPING ADVANCED ANALYSIS TOOLS FOR ZEBRAFISH FACILITY MANAGEMENT
Ana C. Borges
Model Organisms Facility
Gulbenkian Institute of Science (IGC), Oeiras, Portugal

11.15 – 11.20 Discussion

11.20 – 11.25 HOW STANDARDISING LIGHTING IN ZEBRAFISH FACILITIES CAN IMPROVE WELFARE AND REPRODUCIBILITY IN ZEBRAFISH RESEARCH
Paul Tyson[1]-remote, Dave Maley[1] – remote, Gregory Paull[1]
[1]University of Exeter, Geoffrey Pope Building, Stocker Road, Exeter, Devon, UK

11.25 – 11.30 Discussion

11.30 – 11.40 Break

11.40 – 12.30 Global Updates on Zebrafish Research and Resource Centres
Main Chair: Claire Allen **Co- Chair:** Zsolt Csenki-Bakos

11.40 – 11.45 ARCHIVING OF ZEBRAFISH LINES AT THE EUROPEAN ZEBRAFISH RESOURCE CENTER (EZRC)
Robert Geisler[1], Nadine Borel[1], Nathalie Decker[1], Sabine Kaba[1]
[1]Karlsruhe Institute of Technology (KIT), Eggenstein-Leopoldshafen, Germany

11.45 – 11.50 BYPASSING HUSBANDRY: CRYOBANKING OF ZEBRAFISH GERMPLASM – A HUSBANDRY TOOL FOR EVERYONE
Zoltán M. Varga – remote
Zebrafish International Resource Center, Institute of Neuroscience,
University of Oregon, Eugene, USA

11.50 – 11.55 REPORTS FROM THE ANTIPODES. A QUICK ROUNDUP FROM AUSTRALIA & NEW ZEALAND
Bruce Newell[1,2] – remote
[1]Deakin University, Victoria Australia
[2]Australian New Zealand Association of Aquarium Professionals Inc.

11.55 – 12.00 DEVELOPMENT OF ZEBRAFISH RESEARCH AND THE ZEBRAFISH RESOURCE CENTER IN CHINA
Luyuan Pan[1]- remote, Kuoyu Li[1], Yonghua Sun[1]
[1]China Zebrafish Resource Center, National Aquatic Biological Resource Center,
Institute of Hydrobiology, CAS, China

Friday

12.00 – 12.05 WHAT ZEBRAFISH CAN TEL US ABOUT WATER QUALITY OF RIVERS?
Mônica Machado[1]-remote, Laura Lopes[1], Matheus Barcellos[1], Paula Nakamura[1], Aiyra Oliveira[2], Sarah Eibdalla[2], Isadora Figueiredo[2], Isabella Sousa[2], Giovanna Fantin[3], Carolline Porto[3]
[1]Animal Biocience Pos Graduate Program, Federal University of Jataí, Jatai, Brazil
[2]Medicine Veterinary Graduation student, Federal University of Jataí, Jatai, Brazil
[3]Biomedicine Graduation, Federal University of Jataí, Jatai, Brazil.

12.05 – 12.10 ZEBRAFISH IN THE AFRICAN BIOMEDICAL RESEARCH: AN UPDATE
Patrick Amoateng – remote
University of Ghana, Ghana

12.10 – 12.15 UPDATE ON THE SCANDINAVIAN ZEBRAFISH COMMUNITY
Lars Brautigam – remote
Karolinska Institute, Stockholm, Sweden

12.15 – 12.20 UPDATES TO ZFIN: INCREASED SUPPORT FOR TOXICOLOGY AND ENVIRONMENTAL EXPOSURE DATA
Yvonne Bradford – remote
Zebrafish Information Network, Eugene, Oregon, USA

12.20 – 12.30 Discussion

12.30 – 13.20 Career session
Main Chair: Joana Monteiro **Co- Chair:** István Szabó

12.30 – 12.45 STRATEGIES FOR RECRUITING AND RETAINING ANIMAL TECHNOLOGISTS IN A POST-PANDEMIC WORLD
Mollie Millington
Biological Research Facility (BRF), Francis Crick Institute, London, UK

12.45 – 12.50 Discussion

12.50 – 13.00 HARMONISATION OF EDUCATION AND CAREER PATHWAYS FOR LABORATORY ANIMAL CARETAKERS, TECHNICIANS AND TECHNOLOGISTS
Klas Abelson – remote
Department of Experimental Medicine,
University of Copenhagen, Denmark

13.00 – 13.05 Discussion

13.05 – 13.15 HOW TO DEVELOP A TRAINING AND COMPETENCY PROGRAMME ENCOMPASSING AQUATIC SPECIES
Helen Bailey – remote
Biological Research Facility (BRF), Francis Crick Institute, London, UK

13.15 – 13.20 Discussion

13.20 – 14.30 Lunch at exhibition hall

14.30 Closing session + Quiz tombola + facility tours

15.30 – 16.00 facility tour – group 4 (max 15 people)
16.00 – 16.30 facility tour – group 5 (max 15 people)
16.30 – 17.00 facility tour – group 6 (max 15 people)

Posters

1. ESSENTIAL GUIDANCE FOR LARGE-SCALE MANAGEMENT OF THE AFRICAN TURQUOISE KILLIFISH, NOTHOBRANCHIUS FURZERI

Elena De Felice[1], Daniela Giaquinto[2], Chiara Attanasio[2], Antonio Palladino[3], Paolo de Girolamo[2], Livia D'Angelo[2]
[1]School of Biosciences and Veterinary Medicine,
University of Camerino, Camerino, Italy
[2]Department of Veterinary Medicine and Animal Production,
University of Naples Federico II, Italy
[3]Department of Agricultural Sciences,
University of Naples Federico II, Italy

2. ARE SHORT- AND MID-TERM FASTING IMPLICATED IN ZEBRAFISH WELFARE?

Maria Raggio[1], Daniela Giaquinto[1], Chiara Attanasio[1], Antonio Palladino[2], Elena De Felice[3], Paolo de Girolamo[1], Livia D'Angelo[1]
[1]Department of Veterinary Medicine and Animal Production,
University of Naples Federico II, Naples, Italy
[2]Department of Agricultural Sciences,
University of Naples Federico II, Portici, Italy
[3]School of Bioscience and Veterinary Medicine,
University of Camerino, Camerino, Italy

3. DETERMINING THE LINK BETWEEN HORMONES, OPIODS AND TASTE PERCEPTION IN ZEBRAFISH: A PILOT PROJECT WITH IMPLICATIONS IN AQUACULTURE.

Antonio Pallladino[1], Katayoon Karimzadeh[2], Chinelo Uju[2], Paolo de Girolamo[3], Suraj Unniappan[2], Livia D'Angelo[3]
[1]Department of Agricultural Sciences,
University of Naples "Federico II"
[2]Western College of Veterinary Medicine,
University of Saskatchewan
[3]Department of Veterinary Medicine and Animal Productions,
University of Naples "Federico II"

4. POTENTIAL TOXICOLOGICAL EFFECTS OF FUNGICIDES MIXTURES IN ZEBRAFISH EARLY LIFE STAGE

Annamaria Iannetta[1], Tommaso Silvestrini[1], Gabriele Lori[2], Irene Masciola[2], Sabrina Tait[2], Monia Perugini[1]
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5. EFFECTS OF SPIRULIN-SUPPLEMENTED DIET ON ZEBRAFISH: A LONGITUDINAL STUDY

Ferdinando Flagiello[1], Antonio Palladino[2], Stefano Mazzoleni[2], Marcello Diano[3], Maria Raggio[1], Livia D'Angelo[1] Paolo De Girolamo[1]
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6. PHYSICAL AND SENSORIAL ENRICHMENT IN ZEBRAFISH AND MEDAKA: EVALUATION OF PREFERENCES, IMPACTS, AND WELFARE CONSIDERATIONS

Evgenia Dunaevskaya[1], Marco A. Vindas[1], Ian Mayer[1], Romain Fontaine[1], Anthony Peltier[1], Arturas Kavaliauskis[1], Eirill Ager Wick[1]
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7. ARCHIVING OF ZEBRAFISH LINES AT THE EUROPEAN ZEBRAFISH RESOURCE CENTER (EZRC)

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8. ENGINEERED BACTERIOLYTIC ENZYMES AS AN ALTERNATIVE TO ANTIBIOTICS FOR TREATING INFECTIONS IN AQUACULTURE

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9. PAIN IN ZEBRAFISH BEYOND FIN CLIPPING: THE CASE OF THE TRAUMATIC BRAIN INJURY MODEL

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10. ZEBRAFISH HEALTH MANAGEMENT - THE VETERINARY SIDE OF THE STORY
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11. OPTIMIZING ZEBRAFISH HUSBANDRY: ENHANCING EMBRYONIC SURVIVAL AND POPULATION HEALTH IN THE LABORATORY
Martina Bőhmová[1], Andrea Benčeková[1], Peter Fabian[1]
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12. ADDRESSING SEX BIAS IN ZEBRAFISH POPULATIONS: A PRACTICAL ATTEMPT TO BALANCE SKEWED RATIOS
Liliana Vale[1], Maysa Franco[1], Inês Santos[1], André Barros[2], Tiago Paixão[2], Ana Cristina Borges[1], Manuel Rebelo[1]
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13. HOW STANDARDISING LIGHTING IN ZEBRAFISH FACILITIES CAN IMPROVE WELFARE AND REPRODUCIBILITY IN ZEBRAFISH RESEARCH
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14. BEHAVIORAL ANALYSIS OF ZEBRAFISH (DANIO RERIO) LARVAE EXPOSED TO ACESULFAM POTASSIUM AND FLUFENACET
Bence Ivánovics[1], István Szabó[1], Balázs Kriszt[2], Béla Urbányi[3], Zsolt Csenki-Bakos[1]
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15. AUTOMATED IMAGING ROBOT - A NEW INNOVATIVE TECHNOLOGY FOR THE AUTOMATED PHENOTYPING OF ZEBRAFISH EMBRYOS
Illés Bock[1], Jan Sonneville[2], Kees-Jan van der Kolk[2], Urbányi Béla[1], Kriszt Balázs[1], Zsolt Csenki[1]
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16. UTILIZATION OF AFLATOXIN B1 CONTAMINATED CORN BY *TENEBRIO MOLITOR* AND ECOTOXICOLOGY TEST OF BYPRODUCT FRASS BY *DANIO RERIO* EMBRYO TOXICITY TESTS

Zoltán Vajnai[1], Zsolt Csenki-Bakos[1], Cintia Volner[1], Illés Bock[1] and István Szabó[1]
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17. OPTIMIZING ZEBRAFISH SPACE MANAGEMENT: THE ROLE OF SPERM FREEZING AND IN VITRO FERTILIZATION

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18. INTRODUCTION OF A STEP-BY-STEP PROTOCOL FOR THE ERADICATION OF MYCOBACTERIUM HAEMOPHILUM IN ZEBRAFISH SYSTEM

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19. DETECTION OF MICROPLASTICS IN ZEBRAFISH HOUSING SYSTEMS: CAN MICROPLASTIC BACKGROUND CONTAMINATION AFFECT THE FINAL RESULTS OF MICROPLASTIC-RELATED TOXICOLOGICAL TESTS?

Bence Prikler[1, 2], Gábor Bordós[1], Balázs Kriszt[2], Adrienn Micsinai[1], István Szabó[3], Brigitta Nyírő-Fekete[1], Zoltán Palotai[1], Edit Kaszab[2], Sándor Szoboszlay[2], Zsolt Csenki[3]
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ABSTRACTS OF ORAL PRESENTATIONS

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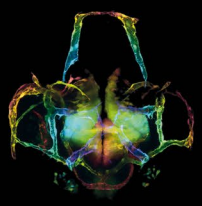

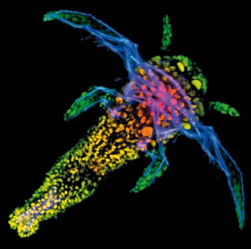

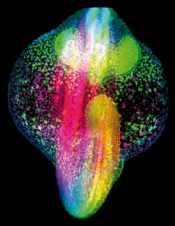

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ENVIRONMENTAL ENRICHMENT

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Living in a barren, monotonous environment causes stress and boredom in captive held animals and this also applies to the laboratory context. Experimental animals exhibit negative signs of welfare when held in barren cages or tanks thus enriching that environment to prevent stress is crucially important. Empirical evidence over many years has shown positive effects of environmental enrichment in zebrafish and taken together these studies suggest the enrichment should be adopted to keep these animals in good welfare. Healthy zebrafish ensure high quality and valid data in research, but questions remain on what type of enrichment should be used and can these be standardised across facilities since this may affect reproducibility between laboratories. Enrichment should also be hygienic, easy and quick to clean as well as inert. The impact of different types of enrichment will be discussed with a view to inspire more research in this area and support the use of enrichment to improve zebrafish welfare.

REDUCING THE ENVIRONMENTAL IMPACT OF ZEBRAFISH FACILITIES

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Considering the environmental impact of laboratory animal facilities has become an ethical challenge. Indeed, projects are approved when benefits to science and medicine are likely to outweigh the harm induced to laboratory animals. However, this forgets to consider the harms induced to the rest of the planet. What if the project would be more environmentally costly than the disease it is aiming to cure? This is an unlikely scenario, but the question should not be left unaddressed.

The aim of the presentation is to empower participants with some resources and basic understanding of key concepts to work on the carbon footprint and environmental impact of their facilities. We will highlight national and international initiatives lead by lab networks, such as labo1.5 organised by French universities, the Laboratory Efficiency Assessment Framework (LEAF) lead by University College London and now used by 85 institutions. Most of these networks are initiated by scientists and are a great way to collaborate between animal facility staff and their users. See also the freezer challenge in which -80°C freezers are kept at higher temperature and the quality of the samples is shared internationally.

We will then deep dive into translation factors for carbon footprint calculation and understand how these vary along the day, week, year, and geographical location. This will take us to two practical applications. The first one will discuss the water renewal in a zebrafish (*Danio rerio*) system, and how to adjust it according to nitrate scores, in compliance with the newly amended directive 2010/63/EU and other recommendations. The second example will show how to use carbon footprint calculation to organise the work pattern for the cleaning and disinfection of tanks in a zebrafish facility and evaluate the impact of autoclaves compared to alternative means of disinfection. With all these points in mind, the participants will be able to look at their internal process differently and find some help to reduce the environmental impact of their zebrafish facility.

GENETICALLY ALTERED ZEBRAFISH AT THE CROASSROAD BETWEEN SCIENCE AND LAW

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2. Federation of European Laboratory Animals Science Associations – FELASA

Genetically altered (GA) zebrafish are valuable models in the study of particular gene functions or when investigating disease mechanisms. The number of GA zebrafish accounts for about 51% out of all other species, as documented by the Summary Report on the statistics on the use of animals for scientific purposes in the Member States of the European Union and Norway in 2022. According to the Directive 2010/63/EU, creation of new GA lines or maintenance of existing GA lines with a harmful phenotype are both within the definition of a procedure, and thus require a project authorization. However, some exceptions exist, for instance the creation of a new GA line by crossing/backcrossing two lines of non-harmful phenotype or the breeding of an existing non-harmful phenotype line. Therefore, the definition of a GA line harmful or non-harmful phenotype is crucial and require transversal knowledge and competences ranging from the method used to generate the GA line to the welfare assessment of the animal in the life course, as well as the legal requirements. The choice of methods of generating new lines of genetically altered animals must be carefully evaluated and justified, since each method offers different challenges and opportunities both for science and about implementation of the Three Rs.
The choice of methods for the genetic characterisation of the line to confirm the desired genotype of the animal at creation, but also to preserve the required genotype with breeding and maintenance, must be accurately selected to reduce welfare constraints.
The welfare assessment pipeline must include key component from the temporal, sample sizing and methodological perspective. At least two age-stages of zebrafish must be considered in the welfare assessment: the point of independent feeding and the stage of adult sexually mature. In case of harmful phenotypes expected to develop at old stages, also older animals should be assessed. Using common welfare assessment schemes helps in the development of consistent approaches to severity classification, in the identification of early time or humane endpoints, to build a quality control process and to early identify relevant deviation from original phenotype which may impact animal welfare and scientific results. Last but not the least, a common welfare assessment scheme is the key to a consistent approach in the welfare evaluation with the final goal of harmonisation across Europe.

USING ENVIRONMENTAL DATA TO DRIVE IMPROVEMENTS IN WELFARE, MANAGEMENT, AND REPRODUCIBILITY

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The zebrafish has risen in prominence to become a major model organism in a relatively short period of time, due to its many innate qualities coupled with major advances in genetic editing, imaging, and other technologies. Despite this tremendous growth, the full potential of the zebrafish to help answer important questions in science has yet to be fully realized. One of its greatest features - its broad tolerance of environmental conditions - is ironically one of the factors that also limits its growth. Because there is no one „best" way to care for and manage the fish, it has never been „necessary" to develop standards of care. Within this ambiguity lies hidden variation that limits the power to make improvements in welfare, management, and reproducibility. The solution to this lies in better utilization of the prodigious amounts of environmental data already being collected every day - with the help of AI and ML - to define conditions that can ultimately lead to performance driven standards. This in turn will lead to better welfare, better science, and better outcomes for all involved.

SEEKING TO BETTER ALIGN THE REGULATION OF ZEBRAFISH RESEARCH WITH WELFARE GOALS

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In the UK, the Animals (Scientific Procedures) Act 1986 regulates procedures carried out on 'protected animals' for scientific research and testing that may cause pain, suffering, distress or lasting harm. The act ensures that research using animals is carried out to high ethical standards and prohibits their use where non-animal models are available. Researchers working with protected animals must provide significant scientific reasoning/rationale as to the benefits their research will bring to the scientific community. They must fully consider the principle of the 3Rs—to reduce, refine and replace the use of animals—in their study programme (Procedure Project Licence; PPL). At an institutional level, a PPL application must be reviewed by an Animal Welfare Ethical Review Board before it is submitted to the Home Office for approval, all of which can take considerable amount of time (i.e. 6-12 months). In Europe, North America and Australasia, legislation and regulations governing the use of animals in research are consistent with those in the UK, but in parts of Asia and the Middle East few, or no, regulations exist.

The UK Home Office also provides standard protocols to incorporate into animal licence applications, such as how long an animal can be kept, but we suggest that some of these protocols may not provide an optimal approach to ensure best animal practice and welfare. We discuss whether the Breeding and Maintenance protocol for genetically modified (GM) zebrafish (a regulated procedure) that stipulates that GM zebrafish are kept to a maximum age of 18 months before termination is in keeping with the welfare/3Rs goals set by the regulators. We highlight the pros and cons of this policy, including its failure to adhere to the precautionary principle for animal protection (potential for causing harm when scientific knowledge is lacking). We explore why this policy is not supported by many researchers and animal care technicians alike and is difficult to implement. Drawing on a recent survey, we will also explore how this policy is not being applied with parity across UK zebrafish facilities, causing confusion for practitioners and likely contributing to data reproducibility problems. We suggest alternative approaches to establishing maximum age profiles for GM zebrafish and how the evidence needed for optimising a 3Rs approach to zebrafish colony management might be best developed. Finally, we reflect on the potential for significantly reducing the need to raise so many zebrafish for research purposes.

PHYSICAL AND SENSORIAL ENRICHMENT IN ZEBRAFISH AND MEDAKA: EVALUATION OF PREFERENCES, IMPACTS, AND WELFARE CONSIDERATIONS

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Environmental enrichment (EE) is a strategy aimed at enhancing the living conditions of captive animals to stimulate their behavior and improve their welfare. In the current study, we conducted two separate experiments to determine the most suitable EE for two model fish species, zebrafish (*Danio rerio*) and medaka (*Oryzias latipes*).
The goal of the first experiment was to investigate zebrafish and medaka preferences for physical or sensorial (colored light) enrichment in the plus-maze apparatus. Initially, place preference was run towards 4 distinct conditions (stones and plants, bottom stones picture, stones and plants pictures or barren) assigned to each maze arm. Lately, four LED-lights (red, green, blue and white) were used as environmental enrichment and placed at the end of each maze arm. Fish preference was analysed by counting an average number of fish in each arm during a five-minute period at different time points. Both species showed a preference for an enriched environment over the barren condition and for the stones and plants pictures over the only bottom stones pictures. Some preference towards the red and white light was observed for zebrafish whereas medaka preferred white and blue light.
During the second experiment medaka and zebrafish were kept in different conditions from the newly hatched larvae until the adult fish. Red or green LED-lights, stones and plastic plants, bottom stones picture, stones and plants pictures, and black bottom were chosen as enrichments. Reproductive traits were used as welfare indicators. Rearing under the green light postponed onset of spawning in medaka and led to a low, but consistent, egg production across replicates and sampling points in zebrafish. At the same time hatching success was higher for the zebrafish eggs from green light enrichment compared to tanks with plants and stones. Deformation ratio was highest in zebrafish larvae hatched from eggs spawned under red light enrichment.
The results may provide evidence about the importance and positive effects of EE on fish behaviour and reproduction, aiding in enhancing fish welfare and increasing research quality. At the same time, it is essential to consider the potential negative impacts of certain enrichments in order to prevent stress and welfare issues during the husbandry of fish.

PAIN IN ZEBRAFISH BEYOND FIN CLIPPING: THE CASE OF THE TRAUMATIC BRAIN INJURY MODEL

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As a laboratory animal, zebrafish may be subjected to several procedures involving pain. In accordance, several studies confirm the need for analgesia for fin clipping, however, there are many other potentially painful procedures for which pain and pain control have not been studied, for example the induction of the traumatic brain injury (TBI) model. In TBI, the zebrafish is subjected to a stab wound head injury. Therefore, our goal was to study pain after TBI induction and if the standard analgesic protocol of lidocaine provided pain relief.
Mixed-sex (1:1 ratio) AB adult zebrafish were randomly distributed into the following groups (n= 5-8): TBI (animals subjected to TBI), TBI+A (animals subjected to TBI and immersed in 5 mg/L of lidocaine for 45 min pre- and for 24h post-TBI), and Sham. TBI was induced by the introduction of the bevel of a 30 G needle in the adult zebrafish left forebrain under anaesthesia (170 mg/L MS222); sham animals received the same concentration of anaesthesia. After the procedures, animals recovered in fresh water (sham, TBI) or in a lidocaine solution (TBI+A). The swimming patterns of all the animals were recorded for 15 min before (baseline), 1h, 3h, 24h and 48h after the procedure.
After analyzing the videos with the Anymaze tracking software, a mixed model with Restricted Maximum Likelihood were used for the statistical analysis. Distance swum, angular velocity and distance swum at the bottom of the tank were significantly different throughout time. Although there were no statistically significant differences between groups, the TBI group was the one with the lowest values of activity, usually associated to animal discomfort.
Behavioural alterations are expected due to brain trauma, however, lidocaine seemed to alleviate the altered behavioural patterns, indicating that the observed changes were probably due to pain rather than to neurological alterations caused by forced trauma. Providing 24h or 48h of analgesia duration did not seem to make a difference. Nevertheless, this is a preliminary study that need to be replicated with a larger sample size.
These preliminary data support the use of analgesia during the TBI procedure. This type of research is crucial for improving the welfare of zebrafish and can offer valuable insights for other invasive procedures in zebrafish where pain is neglected. This study was conducted in collaboration with researchers developing this model, an approach we believe is ideal for improving zebrafish welfare by applying the outcomes in practice.

ZEBRAFISH HEALTH MANAGEMENT - THE VETERINARY SIDE OF THE STORY

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Zebrafish (*Danio rerio*, Hamilton, 1822) have become a pivotal animal model in biomedical research due to their genetic similarity to humans, transparent embryos, and rapid development. The health of the zebrafish is one of the most important aspects of their husbandry. Animals may suffer from several infectious or non-infectious diseases. Prevention should be principal. Infectious diseases can cause significant losses in the stock. The first step to improve biosecurity is to keep newly arrived fish in the quarantine room. During the quarantine period (4 weeks) veterinary investigation is crucial. Viral, bacterial, fungal pathogens and parasites can also be in the background of the zebrafish diseases. Numerous viral diseases are studied in zebrafish model, but there are several (Redspotted Grouper Nervous Necrosis Virus, Infectious Spleen and Kidney Necrosis Virus, Zebrafish Picornavirus, etc.) which may cause mortalities in the stock. Facultative and obligate pathogen bacteria can induce diseases. *Aeromonas* species usually cause diseases as a secondary pathogen, a primary problem is needed to manifest the infection. *Mycobacterium* species can persist in the stock as obligate pathogens and may lead to subclinical infection. However other species are correlated with severe acute to chronic infections, which can cause mortalities. Diagnosis is the most important for bacterial infections. Without information about the disease, without an antibiogram, treatment may be ineffective and contribute to the development of antimicrobial resistance. Fungal infections as *Saprolegnia* sp. can infect even the eggs and the fish too. The hyphae will colonize the eggs in the hatchery and the hatching rate will be lower. The juvenile or adult zebrafish can also suffer from water mold infection on the external surface and on the gills, but a primary cause is needed. External and internal parasites are affecting the zebrafish too. Ciliates, like *Ichthyophthirius multifiliis* can damage the gills and the skin, which can lead to secondary infections and oxygen deficiencies. *Transversotrema* sp. attaches to the skin, and the fish will scratch themselves on the wall and at the bottom of the tank. Heavy infection can cause stress and mortalities in the stock. Parasites from higher taxa, like *Pseudocapillaria* sp. live in the intestine of the zebrafish and the eggs of the worms can be found in the fecal samples of the fish. Treatment is possible after the right diagnosis. Proper husbandry, strict biosecurity standards and veterinary control are essential to maintain the stable stock needed for successful biomedical experiments.

IMPLEMENTING THE 3R UNDER THE AMENDED DIRECTIVE 2010/63/EU

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Directive 2010/63/EU has seen a few developments in recent years, and some were zebrafish (*Danio rerio*) specific. First the guidance on the breeding and reporting of zebrafish with (non)-harmful phenotypes clarified a few points of discussion, such as adult fin clipping being a regulated procedure and requiring analgesia. Welfare assessments and the need to monitor fish welfare with the help of scoresheets is another topic for which resources have been created. We will propose some practical examples of applications for these refinements, and this will lead to examples on severity assessments. This year, the directive was amended to set by the end of 2026 some strict requirements on zebrafish housing. These have practical applications which will promote 3R implementations. To discuss these, we will travel with the participants from the husbandry of the past to the future routine practices. For example, the new requirement on tank sizes will directly impact the housing of adult zebrafish post fin clipping, and it will therefore promote genotyping methods at larval developmental stages. Housing fish for breeding will also be affected to some extent. The amendment of the directive also sets some standards for adult fish density and water parameters, and we will see how to comply with those.
A major regulatory change will be the authorisation of hypothermic shock to euthanise adult zebrafish. This is a more difficult topic since the authorisation does not mean that the method is the most refined one. It means that it is as refined as an overdose of anaesthesia, bearing in mind that the law does not consider which anaesthetic to use to induce this overdose. Therefore, we will discuss experimental data and the FELASA recommendations to choose the right method of euthanasia according to the context, e.g. whether samples of a certain quality are required or not.

EFFECTS OF *SPIRULIN*-SUPPLEMENTED DIET ON ZEBRAFISH: A LONGITUDINAL STUDY

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In aquaculture and zebrafish breeding for scientific research, feeding strategies are crucial having effects on the animals' welfare, growth, and reproduction, as well as significant economic and environmental impacts. Recently, Spirulin has emerged as powerful supplementation in fish due to the rapid and easy cultivation in bioreactors and excellent nutritional profile. Literature indicates that the effects of spirulina on fish growth vary with the supplemented amount and are species-specific. Considering this, we conducted a 32-weeks longitudinal study on adult zebrafish, daily fed with a food/spirulina ration:

- Group 1: 100% standard feed
- 2: 100% spirulina
- 3: 50% standard feed and 50% spirulina
- 4: 75% standard feed and 25% spirulina
- 5: 25% standard feed and 75% spirulina
- 6: 95% standard feed and 5% spirulina

Starting from the second week of supplementation, we evaluated the biostimulant effects on fitness and reproductive performance of all groups. At the end of the period, we conducted a morphological study on male and female gonads.

Regarding fitness, we evaluated survival, body condition index (BCI), and Specific Growth Rate (SGR). We used "Body condition scoring 2 in the scale defined by Clark et al 2018 [1] as the humane endpoint for the fish in the study. Expectedly, animals of group 2 showed the worst performance. They reached BCS 2.I, and were therefore euthanized, before the 10th week of the study, since spirulina does not provide the adequate macro- and micro-nutrients required for a fish diet. Animals of the group 6 and 4 displayed the highest survivorship in comparison with the other groups. BCI calculation revealed that all groups were within the physiological range over the experimental period. Remarkably, group 6 had a higher BCI from the tenth week consistently with higher SGR in the early weeks.

Parameters to evaluate the reproductive fitness *in vivo* were: 1) number of eggs produced per mating, 2) number of fertilized eggs, and 3) total number of hatched larvae. Only group 4 gave results comparable with the control group, differently from the other groups.

In the morphological study, we identified and counted the number of pre-vitellogenic and vitellogenic eggs, with groups 1 and 4 having similar quantities of pre-vitellogenic and vitellogenic eggs, in the other groups, more post-vitellogenic eggs were detected, accounting for 83% in group 6 particularly. In the

male gonads, we counted the number of spermatogonia, spermatocytes, and spermatozoa, observing that in group 6 more spermatogonia out of the total identified cell types were observed in comparison to other groups, thus indicating that more precursors of germ cells are present in this group and that could represent an advantage in long lasting reproductive capabilities. In groups 4 and 5 spermatocytes were the largest number of identified cells, while in groups 1 and 3 more spermatozoa over the other cell populations were counted.

These preliminary results shed light on the potential effects of lifelong supplementation of spirulina, generally used as biostimulant and healthy improvement for a very restricted period. It emerges that spirulina can be incorporated into the diet for an extended duration, with the best growth results achieved at a 5% integration, while optimal reproductive outcomes occur with a 25% integration.

References: Clark, Tannia S., et al. “Body condition scoring for adult zebrafish (Danio rerio).” Journal of the American Association for Laboratory Animal Science 57.6 (2018): 698-702.

Fundings: Noself srl - www.noself.it

ARE SHORT- AND MID-TERM FASTING IMPLICATED IN ZEBRAFISH WELFARE?

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Fasting triggers metabolic responses in organisms and influences several cellular pathways, impacting overall health and welfare. Understanding these effects is crucial for improving animal welfare, management and husbandry practices, particularly in research settings as well as in aquaculture.
In this study, we explore the zebrafish neuronal responses induced by short- and mid-term fasting, focusing on the phosphorylation of ribosomal protein S6 (pS6), a marker of neuronal activity. This timing, respectively to four and seven days of complete food withdrawal, is generally referred in routinely zebrafish practices, *i.e.* genotyping and or transportation.
The experimental design was approved by the Italian Ministry of Health n° 291/2022-PR. Adult fishes (n=10/group – mixed sexes) were divided in three experimental groups: control, four and seven days of fasting. Control group was feed twice/day with SDS (Special Diets Service) 400, a specific aquatic diet for regular maintenance. The four- and seven-days fasting groups were completely food deprived. At the end of the experimental period, animals were euthanized, brains were sampled for western blotting and immunohistochemical analyses.
Western blot results revealed a significant increase in S6 phosphorylation in the seven-day fasting group compared to the control, indicating increased neuronal activity. In contrast, a decrease in S6 phosphorylation was observed in the four-day fasting group. Immunostaining experiments confirm that the highest immunoreactivity occurred upon seven days of fasting in the zebrafish brain with a strong positivity in the neuronal cells of the dorsal telencephalic areas and in the preoptic area. Less numerous and weakly stained neurons were seen in the hypothalamic area, near the hypothalamic recess.
In addition, we detected pS6 immunoreactivity in sensory cells of the taste buds across all groups, identified by immunofluorescence staining.
Our data provide useful insights on zebrafish welfare: seven days of fasting should be carefully considered when planning experiments and take care of the routine management of the fish, differently from four days of fasting which were characterized by the absence of neuronal activation.

THE 3RS IN ZEBRAFISH RESEARCH

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The 3Rs (replacement, reduction and refinement) are an ethical framework embedded in international law and used to guide procedures involving animals in scientific research. In the last decade there has been a significant increase in the use of zebrafish in bioscience research, highlighting the need to promote the importance of applying the 3Rs to experimental studies that use zebrafish as a model organism. In applying the 3Rs to zebrafish research, replacement may involve using mathematical or computer modelling as a complete replacement of the use of zebrafish, or alternatively using embryonic or foetal forms of zebrafish as a form of partial replacement. Refinements in zebrafish research involve any method or approach which can improve welfare or reduce potential harm to zebrafish. Any refinements must be robustly validated and designed to encourage engagement in zebrafish-specific behaviours. It is therefore essential for researchers and facility staff to be adequately supported when implementing refinements, particularly when there is a strong evidence base which demonstrates the potential benefit to the quality of lives of laboratory zebrafish. Finally, reduction in zebrafish research refers to using the most appropriate number of zebrafish required to obtain statistically meaningful results, and using methods and techniques to minimise the use of zebrafish where possible. This may involve using appropriately designed studies that maximise the amount of information obtained per zebrafish, and detailed reporting of experiments to improve study reproducibility. To assist in the design and reporting of scientific experimental studies which involve the use of zebrafish, and vertebrates more broadly, resources and guidelines have been developed over the last decade. This talk will introduce the 3Rs and will discuss how embedding them in zebrafish research ensures that the highest standards of welfare are applied. In applying the 3Rs to zebrafish research, scientific progress can be advanced through better reporting, more reproducible studies, and the development of new methods and technologies.

WELFARE ASSESSMENT, PAIN AND ANALGESIA

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Pain in laboratory animals is problematic for many reasons especially when it reduces the welfare of the animal and may confound experimental data if researchers are recording the response to pain rather than response to the treatment. From an ethical and legal perspective, we must ensure pain is avoided or minimised in experimental animals including zebrafish. How to recognise pain as well as alleviate it with use of pain-relieving drugs is vitally important. Examples of pain-related behaviour will be discussed alongside pain management protocols which are important Refinements under the 3Rs. The recommendations from a recent FELASA report provide invaluable information on pain management strategies that allow the Refinement of common laboratory procedures in zebrafish.

HARMONISING RESEARCH FOR THE 3RS: TAILORING ZEBRAFISH HUSBANDRY FOR THEIR PURPOSED RESEARCH.

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Zebrafish are often described as an easy to keep species, and by the Tropical Fish Hobbyist magazine as “a perfect beginner fish”. This, however, is far from being the case when keeping this species in the laboratory for different and diverse research purposes and needs. In the first part of this talk, we will describe the various challenges faced for the application of zebrafish in the research areas of behavioural neuroscience, epigenetics and ecotoxicology. We will then illustrate, with examples from the literature, how the lack of standardisation of husbandry practices/approaches in the zebrafish research community is contributing to uncertainties in research data and in some cases contributing to poor experimental reproducibility. The growing body of evidence linking zebrafish husbandry practices with problems in data reproducibility leads us to call to the ‘zebrafish community’ to help develop current husbandry practices, which are often in the most basic form, to better suit their research use and the animal’s welfare. Finally, we will discuss some ideas/concepts and identify existing expertise to help achieve practical developments in zebrafish husbandry that better align with their research use.

REDUCING SEVERE SUFFERING IN REGULATORY TOXICOLOGY TESTING USING FISHES

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Mortality is a key cause of severe suffering, and there can be an increased risk of this in tests using fishes, e.g. in regulatory toxicology procedures within OECD 203 and OECD 210 testing guidelines. In November 2023, the RSPCA organised an in-person meeting focusing on the application of humane endpoints in regulatory toxicology studies that use fishes, with the aim of identifying and sharing strategies to reduce and avoid severe suffering. The meeting also explored some of the challenges associated with this goal. Two of the main conclusions from the meeting centred on standardising approaches to (i) identifying sublethal clinical signs and applying humane endpoints, and (ii) improving staff training around identifying clinical signs, fish behaviour and welfare.

As a result of the meeting, the RSPCA has produced a report with specific recommendations for the wider scientific community, including regulators, scientists, animal technologists and unit managers, and training organisations. This talk will explore these recommendations and discuss how progress could be made towards reducing severe suffering in regulatory toxicology.

PISCINE ENDPOINTS IN EXPERIMENTS AND THE SIGNIFICANCE OF USING SCORE SHEETS TO REGISTER 'EVERYDAY DISORDERS' IN FISH

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Fish are the most used research animals in Norway. Although improvements have been made in recent years when it comes to fish welfare in Norway, the focus has mainly been on farmed salmonids, not research animals. In addition, the focus has been on preventing and combating diseases causing large economical losses to the salmon farming industry. Diseases or minor lesions that do not lead to mortality or to reduction in production quality has not been given the same attention. Smaller welfare issues, such as abrasions, minor fin lesions or operculum shortenings are all a part of what can be considered the fishes' 'everyday disorders', problems that the fish will be able to cope with, but that might affect growth, performance and ultimately the fishes' welfare. Lesions affecting various body parts are evaluated according to several categorical scales and score systems (welfare indicators). Most of these, however, are based on evaluation of euthanized fish, i.e. fish out of water, and not live monitoring of fish. Score sheets can be important tools to monitor, standardize and document fish welfare in studies. Using score sheets to detect any subtle changes in morphology or behaviour may help develop knowledge about why these `everyday disorders` occur and how these might affect fish welfare. Examples of specific morphological piscine endpoints for lesions often seen during various experiments will be shown together with examples from some of the most common minor lesions seen in salmon in challenge facilities.

GENOTYPING WORKSHOP

Lisa Van Hateren[1]

1. The University of Sheffield

Genotyping zebrafish is an essential process to identify mutant alleles within research facilities. Fin clipping of adult zebrafish has been the standard genotyping method employed by many institutions for several years. More recently, alternative genotyping methods have been explored to help reduce the number of protected animals and refine procedures as part of continual 3Rs improvements. In this workshop we evaluated some of these methods to understand why adult fin clipping is still predominantly used in facilities rather than alternative methods, given the importance of the 3R's.

SKIN SWABBING: A REFINEMENT FOR DNA SAMPLING OF LABORATORY FISH

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Small fish species are commonly used as experimental models in the laboratory. DNA is routinely collected from these animals for genotyping. The current standard procedure to for this is fin clipping, by anaesthetising individuals and removing a portion of the caudal fin. Fin clipping reliably generates good quality DNA samples for downstream applications, however evidence shows it can impact fish health, welfare and behaviour. This can result in greater variation in the data collected. In a recent study we adapted a skin swabbing protocol to collect DNA from small-bodied fish, including sticklebacks and zebrafish, without the use of analgesics, anaesthetics or sharp instruments. A rayon-tipped swab was used to collect mucus from the flank of the fish, which was then used for DNA extraction. We subsequently demonstrated that skin swabbing triggered fewer changes in stress axis activation and behaviour when compared to fin clipping. We also found that gene expression and behaviour data collected from swabbed fish were less variable than similar data collected from fish that had been fin clipped. This potentially allows smaller sample sizes in experimental groups to be used after skin swabbing, thereby reducing animal use.

IDENTIFICATION OF INDIVIDUAL ZEBRAFISH: A REFINED PROTOCOL FOR VIE TAGGING WHILST CONSIDERING ANIMAL WELFARE

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Zebrafish are an important model system for scientific and medical research. Despite this, marking zebrafish for individual identification purposes is not commonplace. In other fish species, visible implant elastomer (VIE) tagging is used as a successful identification method but lacks important details regarding fish welfare. We highlight previously unconsidered animal welfare issues through long-term observations of survival rate, tag retention, and tag colour on different populations and age-groups of zebrafish; and introduce an improved VIE tagging protocol. This improved protocol was developed and compared with original tagging procedures and associated negative effects, following animal welfare concepts described in the Three Rs principles, focusing on Refinement. We describe a novel protocol using lidocaine solution as an analgesic and post-tagging treatments with two healing agent to improve the wound healing. The information from this study will be beneficial for the zebrafish research community as a guideline for implementing VIE tagging as a successful identification tool when differentiating between genetic lines, families, or individuals. And will also be beneficial for the whole fish biology community when considering important animal welfare questions in the future if they are using identification techniques which could be considered as potentially noxious stimuli for fish.

IMAGING WORKSHOP

Claire Allen[1]
1. Biological Services Aquarium (BSA) Faculty of Science, University of Sheffield, UK

Zebrafish are an excellent model for imaging, with unparalleled potential to analyse sub-cellular processes at a detailed level. With the correct microscopes, researchers can aim to view any stage of development in a visually accessible vertebrate. This can be at a range of magnifications, over a long period of time and with the option of deep-tissue imaging. These data sets can be collected over the life course of the fish. The focus of the workshop is practical management of live imaging husbandry both for embryonic and adult stages. The importance of technical experimental ability in imaging cannot be underestimated. The workshop will adapt to the demands of the audience, starting with a description of the various standard microscopy techniques. This will be followed by examples of methods and protocols used for different types of imaging. The welfare and husbandry of zebrafish is of huge importance for all experimental design. It must be robust enough not to impact upon the results being collected. All researchers must understand that a happy fish is a fish that will give you the most robust scientific data. A sick fish will only give you data relating to that biological state or disease-stage. Ultimately we need to challenge what we see and ensure reproducibility. By optimising sample preparation with competent microscopy, data produced will be interrogatable.

THE REPRODUCIBILITY CRISIS IN ZEBRAFISH RESEARCH – WHY NOT TELLING IS ONLY AN OPTION IN LAS VEGAS

Karin Finger-Baier[1]
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Research with zebrafish, as with other model organisms, suffers from reproducibility issues. Experimental outcomes cannot be replicated within labs or across institutions and published results cannot be reproduced based on the methods provided. But why? Are our technical skills inferior to those of our colleagues, collaborators or competitors? Perhaps not. Perhaps it's bits of crucial information missing in these protocols and publications. These may be related to husbandry parameters, experimental design, the model itself or data analysis, among others.
We will look at various types of information that are at risk of being omitted, with the focus on internal state of the animals (including circadian rhythm) and husbandry parameters. We will also briefly discuss how to address the forgotten facts and how to add them best to protocols and publications.

ROLE OF WATER CHEMISTRY IN ZEBRAFISH WELFARE AND REPRODUCIBILITY OF RESEARCH STUDIES

Rod W. Wilson[1], Cosima Porteus[2], Jennifer Finlay[1], William Davison[1], Simon MacKenzie[3], Ella Waples[1], Madeleine Calvert[1], Anna Dempsey[1], Alex Harber[1], Aislin Chambers[1], Gregory Paull[1]
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Water chemistry varies hugely across zebrafish facilities often simply depending on the local tap water supply (e.g. physiological important Na+, Cl- and Ca2+ vary by several thousand-fold; Porteus et al., 2024), with no guidelines on many parameters relevant for health. Even for artificial 'freshwater' recipes developed specifically for zebrafish research (e.g. OECD, ISO, E2, E3, Danieau's) sodium varies by >375-fold. Moreover, CO2 is rarely measured, but affects acid-base physiology, behaviour and growth of many fish and in zebrafish facilities can be 7 times higher than atmospheric (400 µatm), and 3 times higher than climate change predictions for end of this century (~1000 µatm). We therefore investigated how freshwater salinity (0.08 to 2 ppt) and CO2 (400 to 4,000 µatm) influenced zebrafish development, growth, behaviour, immune function, swimming performance, post-exercise acid-base regulation, and reproduction. Up to 4 dpf no treatments had any effect on the major developmental milestones (tail detachment, somite formation, hatching, heart beat and touch response) but embryos were ~10 % larger in highest salinity and lowest CO2. However, rather than smaller fish catching up growth once exogenously feeding, zebrafish in the highest salinity were 4-fold larger at 18 dpf, with a smaller tendency for the lowest CO2 level to promote growth. During a whole life cycle study, major differences were caused by both salinity and CO2 in behavioural responses of adults following transfer to a novel tank arena. Maximum swimming speed was also directly proportional to salinity, probably related to gill morphology and area for gas exchange. Spawning success was also impacted with zebrafish from one treatment having no viable eggs. Such differences in growth, anatomy, physiology, behaviour and reproduction of zebrafish of the same age under differing water chemistry regimes are likely repeated throughout the global zebrafish research community, contributing to welfare issues and the reproducibility crisis.

Porteus, CS, Waples, E, Dempsey, A, Paull, G, Wilson, RW (2024). A survey of water chemistry used in zebrafish facilities and their effects on early zebrafish development. F1000 Research, https://doi.org/10.12688/f1000research.134520.1

DIETARY REQUIREMENTS FOR ZEBRAFISH

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Zebrafish have a natural ability to feed on a wide variety of prey with different nutritional content. Historically, facilities have taken that as an advantage for the models' husbandry that allowed feed choice to include other criteria besides nutritional content, namely the facility budget, regional product availability, and workload involved in the feed preparation and distribution. Different feeding routines can also produce satisfactory welfare indicators, such as survival, growth, breeding performance. Nevertheless, this apparently harmless heterogeneity has proven a liability for the scientific outcomes based on the Zebrafish model. Diet affects physiology and behavior, and can interfere with experimental outcomes in uncontrolled ways. Standardisation would improve the reproducibility and comparison of results obtained by different laboratories. Accurate scientific results are key to translate research into products and approaches for human benefit.
We have performed a literature review and present data on the feeding ecology, anatomy and physiology of zebrafish, their nutritional requirements throughout life and how the quality and quantity of different nutrients may affect them. We will focus on the discussion of available feeds and feeding routines, and which seem to be the more consensual approaches worldwide.

RUNNING A ZEBRAFISH CRYOPRESERVATION AND REDERIVATION SERVICE

Helen Horsler[1], Hollie Lane[1], Katharine Crawley[1], Mollie Millington[1]
1. The Francis Crick Institute, London, UK

Zebrafish are a staple model organism in scientific research, and the advancement in genetic engineering tools means there are thousands of lines being produced. Maintaining these lines as living stock when a line is not in use is costly, and goes against the 3Rs principle to reduce animal usage. Without a cryopreserved stock there is a risk of a line being lost due to sickness, natural disaster or genetic drift. Cryopreservation of spermatozoa in zebrafish is an essential tool in the management of stock, allowing for lines to be archived, distributed and rederived when needed. Unlike mammalian species such as mice, due to the size of the zebrafish egg, archiving of zebrafish lines must be achieved solely through sperm cryopreservation. There are several different methods available to freeze down sperm, finding the right one can be challenging. Losing samples due improper cryopreservation or thawing methods can lead to lines being lost, and therefore a robust QC process is key.
In this talk I will be discussing methods using the cryoprotectant N,N-dimethylacetamide (DMA) with a buffered sperm motility-inhibiting solution (BSMIS). This method, combined with terminal cryopreservation, allows for a high throughput service I will outline storage considerations and sample management, as well as an achievable quality control programme to ensure good stocks. Finally I will discuss the revival of frozen stocks via In vitro fertilisation and the considerations that should to be made to ensure new stocks are the correct genotype and health status for your facility.

AGEING

Claire Allen[1]

1. Biological Services Aquarium (BSA) Faculty of Science, University of Sheffield, UK

Ageing is an important husbandry consideration in zebrafish management, but what age is old? It all depends on the fish, the strain, the environmental conditions and the stressors they encounter throughout their life course. There are a number of specific lifestyle regimes associated with ageing in zebrafish, so our traditional view of ageing as a constant with the passing of time is not necessarily an indicator of frailty. Ageing diminishes fecundity, creates tissue damage and reduces physical and mental capabilities. This, in turn, can lead to acceleration of disease prevalence.

A balance is required within zebrafish facilities to ensure aged fish are culled before frailty develops but not so early that fish are still in the prime of their breeding life. The latter scenario can have detrimental impacts on the 3Rs, particularly reduction.

The zebrafish is a good model for ageing since it recapitulates human processes and, as in humans, telomerase deficiency speeds up ageing. In a facility where a major research objective is to elucidate the process of ageing, the aquarium team is required to care for aged fish under protocol. This work has helped to develop a deeper understanding of the process and how to identify old age with more defined endpoints.

In this talk I will discuss research that has revealed clear indicators of ageing, how husbandry can alter the timeline of zebrafish getting old and when to end the protocol.

DEVELOPING ADVANCED ANALYSIS TOOLS FOR ZEBRAFISH FACILITY MANAGEMENT

Ana C. Borges[1], Maysa Franco[1], Liliana Vale[1], Inês Santos[1], André Barros[2], Tiago Paixão[2], Manuel Rebelo[1]
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Zebrafish research is critically dependent on maintaining essential metrics such as breeding performance, survival rates at various life stages, sex distribution, and overall health status. Accurate and consistent records of these metrics are vital for ensuring research reproducibility and the integrity of experimental outcomes.
As zebrafish facilities expand their collections of transgenic lines, managing and effectively utilizing the growing volume of recorded data becomes increasingly challenging. To address these complexities, the Model Organisms Facility has partnered with the Advanced Data Analysis Facility to develop a digital tool designed to streamline the analysis of facility-collected data.
This tool is tailored to analyze key variables indicative of nursery care and colony management, including survival/mortality rates, age of sexual maturation, and sex ratio. By enabling trend assessments, anomaly detection, and line-specific characterization, the tool enhances data-driven decision-making in zebrafish research facilities.
Preliminary results have already provided valuable insights. For example, the tool has identified trends in sex-ratio imbalances across specific zebrafish lines, which can inform breeding strategies and colony maintenance. Additionally, by characterizing line-specific survival rates, the tool highlights lines requiring particular care and attention, potentially improving animal welfare and facility efficiency.
As we continue to develop and refine the tool, our goal is to make it accessible to the wider research community. By presenting these initial findings to experts in the field, we hope to gather critical feedback to guide future improvements, ultimately contributing to the broader field of zebrafish research and facility management.

HOW STANDARDISING LIGHTING IN ZEBRAFISH FACILITIES CAN IMPROVE WELFARE AND REPRODUCIBILITY IN ZEBRAFISH RESEARCH

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Standard tank racking for housing zebrafish in research facilities does not include above tank lighting and, as such, tank illumination is reliant on the wider room lighting alone. This design means that tanks on the upper shelves are exposed to the brightest lighting, whilst those on the bottom shelves are exposed to the lowest level of lighting. At Exeter, we found that this resulted in an 18-fold difference in the Lux (light intensity) levels that zebrafish were exposed to.

The lack of standardisation in lighting for zebrafish research is surprising given that many studies have reported on the importance of the type of lighting on a range of zebrafish physiological and welfare needs as well as scientific research outcomes (including reproducibility). It has been reported that lighting regimes can impact every life-stage of zebrafish from hatching, growth, development, spawning and behaviour. Furthermore, certain types of lighting conditions can even induce stress and inflammatory responses.

Poorly lit tanks also make it difficult for animal care staff and researchers to accurately assess the health and wellbeing of zebrafish under their care and this is especially the case for larval and juvenile zebrafish which, where, due to their small size, health problems are more easily missed during routine health checks.

At Exeter, we are working with, both, Tropical Marine Centre (lighting specialists for fish) and Tecniplast (zebrafish housing specialists) to retrofit an above tank lighting solution to our existing tank racks and thus provide standardised lighting to all tanks and fish irrespective of where the tanks are placed in the room. The poster we present today highlights the progress we have made with this project alongside some of the challenges and considerations faced along the way.

ARCHIVING OF ZEBRAFISH LINES AT THE EUROPEAN ZEBRAFISH RESOURCE CENTER (EZRC)

Robert Geisler[1], Nadine Borel[1], Nathalie Decker[1], Sabine Kaba[1]
1.Karlsruhe Institut of Technology (KIT), Eggenstein-Leopoldshafen, Germany

Laws on animal experimentation aim to reduce the use of animals in European research, stimulating the use of alternative models such as zebrafish embryos. Centralized archiving of genetically modified zebrafish strains can make critical contributions to this aim, since it avoids redundant experiments and improves reproducibility of experimental results. The European Zebrafish Resource Center (EZRC) at the KIT archives 27,000 knock-out mutations from the Sanger Institute (representing nearly half of all protein-coding genes), 2,000 mutations from forward-genetic screens at the MPI for Developmental Biology, and approximately 1,000 mutant and transgenic lines contributed by the community. It also serves a source for standard wildtype lines.
Genome editing by CRISPR can easily produce new mutant lines, however each CRISPR experiment requires a permit, molecular biological characterization of the mutations and evaluation of animals for harmful phenotypes. Therefore we offer free archiving of established CRISPR lines as frozen sperm together with the corresponding severity assessments.
As a further contribution to standardization in the zebrafish field we provide hands-on training in cryopreservation methods and participate in the International Zebrafish and Medaka Courses (IZMC) held several times per year at the KIT.
Finally, European animal health legislation imposes certain requirements on shipping zebrafish embryos or adult fish. We will discuss how these are implemented at EZRC.

BYPASSING HUSBANDRY: CRYOBANKING OF ZEBRAFISH GERMPLASM – A HUSBANDRY TOOL FOR EVERYONE

Zoltán M. Varga[1]

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Public research funding agencies typically require that genetic resources developed with public funds be made publicly accessible for future research and development. However, the rapid increase in novel zebrafish (Danio rerio) lines generated annually by biomedical research is straining the Zebrafish International Resource Center's (ZIRC) cryobanking capacity. While both published and unpublished lines—those still under investigation or reserved for future study—require long-term preservation, ZIRC's mandate primarily focuses on preserving and distributing published, publicly available lines.
To address this, the zebrafish research community needs to transition from a centralized, ZIRC-dependent model to a decentralized, community-driven cryobanking strategy, still supported by ZIRC. While ZIRC will continue to manage the sharing of publicly funded lines, research labs will preserve their unpublished ones. This transition faces two major challenges: the widespread lack of cryopreservation skills and infrastructure within 99% of the aquatic research community, and the absence of standardized quality management. The perception that cryopreservation is difficult further complicates efforts to safeguard zebrafish lines developed with significant public investment. These issues hinder collaboration, jeopardize the preservation of valuable lines, and create inefficiencies that slow future research.
In response, ZIRC, in collaboration with the Aquatic Germplasm & Genetic Resources Center (AGGRC), proposes an interdisciplinary approach to develop a decentralized cryobanking system. This approach will equip individual laboratories with the necessary protocols and tools for reliable sperm cryopreservation and quality management, facilitating coordinated preservation and material exchange within the zebrafish research community.
In this presentation, I will present many benefits of cryopreservation for research laboratories and outline the program we are developing with the AGGRC for the research community.

REPORTS FROM THE ANTIPODES. A QUICK ROUNDUP FROM AUSTRALIA & NEW ZEALAND

Bruce Newell[1,2]
1. Deakin University Victoria Australia
2. Australian New Zealand Association of Aquarium Professionals inc

Bruce Newell has been working in the Zebrafish husbandry field for the last 16 years, after a similar period self employed in the ornamental aquarium trade.
Bruce has previously held multiple positions within the Australian New Zealand Association of Aquarium Professionals inc. (ANZAAP), currently serving in the role of Vice President again.
Bruce will be presenting on the progress and facility developments that are occuring in the Australia New Zealand region, including updates on the ANZAAP Aquatic Symposium for 2025.

DEVELOPMENT OF ZEBRAFISH RESEARCH AND THE ZEBRAFISH RESOURCE CENTER IN CHINA

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Compared with European and American laboratories, zebrafish research in China started late. At the beginning of this century, the number of zebrafish papers published by Chinese researchers accounted for less than 1% of the global total. However, over the past two decades, zebrafish has become one of the most popular model organisms in China, and has been widely used in life, health, environmental and agricultural sciences, etc. According to the core collection in web of science, the global share of total papers by Chinese zebrafish researchers has exceeded 40% by 2023. The China Zebrafish Resource Center (CZRC) was established in 2012 and located in the main campus of the Institute of Hydrobiology, CAS. CZRC receives financial support from the Ministry of Science and Technology of China and the Chinese Academy of Sciences. The research resources available at CZRC include wild-type, mutant, transgenic, and knock-in zebrafish lines, zebrafish cell lines, tool plasmids and antibodies. Now CZRC has more than 3,000 zebrafish lines, 90% of which originated from laboratories in China, and about 30% of the lines were constructed by CZRC locally. All fish lines are rigorously genotype verified and sperm cryopreserved. CZRC adopts a demand-driven culture approach, whereby any zebrafish line that has been required in the last two years is kept in live stock, and now main fish room cultures about 300 live lines. Besides, CZRC also serves the zebrafish research community with technical support, including constructing mutant, transgenic and knock-in lines, diagnosing and disposing of fish diseases, and organizing 2-3 training workshop on experimental techniques for zebrafish every year. The support of these resources and technologies has played a great role in advancing the rapid development of zebrafish-related research in China.

WHAT ZEBRAFISH CAN TELL US ABOUT WATER QUALITY OF RIVERS?

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When it comes to water quality, physico-chemical parameters are the first to be cited as important for the survival of embryos and fish, but the variation seen, especially in large bodies of water, is minimal and other metrics for identifying water quality are important. Ecotoxicology studies can assess the impact of contaminants on the communities of aquatic ecosystems, and the use of zebrafish embryos is a low-cost option, highly sensitive, and allows the identification of multiple lethal, sublethal, and teratogenic factors. The Fish Biotechnology and Physiology Laboratory (LABFISH) has developed studies focused on aquatic ecotoxicology using the zebrafish model, identifying the interference of toxic factors on embryonic development and correlating this with the diversity of the ichthyofauna. The fish are kept in a recirculating water system with temperature and light control, and dissolved oxygen, hardness, pH, nitrite, nitrate and ammonia are monitored. Water samples are collected at 27 points in the Paranaíba River, which is part of the Upper Paraná Basin. The embryotoxicity tests are chronic, with embryos exposed for 196 hpf. The endpoints for toxicity assessment are: heart rate, involuntary movements, survival, teratogenicity, and larval morphometry at 196 hpf. For larval morphometry, three types of measurements were identified: sensory, which identifies eye area and minimum and maximum interocular distance; physiological, based on morphometry of heart area, yolk sac and swim bladder; skeletal, with measurements of head height, head width and head depth. The results indicate that the Paranaiba River is extremely anthropized, however the physico-chemical parameters of the water are in line with the requirements of the fiscal authorities of brazilian water quality guidelines and are suitable for consumption. The point farthest from the hydroelectric plant (mouth of the Aporé River) is the one with the highest mortality rate. Observing the embryonic morphometry, it is clear that the points with high survival rates also allow the formation of healthy larvae. The points with an intermediate mortality (below 70%) promote sensory and cardiac alterations that reduce both the survival and health of the larvae. These results show the importance of water quality, which influences not only embryonic development but also the formation of larval structures. In facilities, physico-chemical quality parameters are closely monitored because they are linked to well-being of fish, but there are doubts about the influence of other factors on embryonic development in zebrafish. It is also important to note that in some cases the physical and chemical parameters allow a high survival rate of larvae, but may affect the morphology of the fish, which could lead to fragility in future generations of the Biotherium.

ZEBRAFISH IN AFRICAN BIOMEDICAL RESEARCH: AN UPDATE

Patrick Amoateng[1]

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At the 2022 ZHA conference, I presented on the utilization of zebrafish in biomedical research across Africa. This presentation will detail the advancements made in promoting zebrafish as a model organism, particularly in Ghana and other African nations. Furthermore, it will discuss the establishment of the Zebrafish Africa Network (ZeFAN) and showcase research findings from zebrafish-based studies conducted throughout the continent. The presentation will conclude with an outline of future initiatives.

UPDATE ON THE SCANDINAVIAN ZEBRAFISH COMMUNITY

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During my presentation, I will give you an update on the recent activities of the Scandinavian zebrafish community and present the newly established Nordic Zebrafish Network.
Amongst others I will give you an overview of our efforts to standardize and harmonize education, husbandry and experimentation as well as how we communicate and raise awareness of the public for the zebrafish animal model and its importance for basic and preclinical research.

UPDATES TO ZFIN: INCREASED SUPPORT FOR TOXICOLOGY AND ENVIRONMENTAL EXPOSURE DATA

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The Zebrafish Information Network (ZFIN, zfin.org) is the database resource for genetic, genomic, and phenotypic data that result from research using *Danio rerio*. ZFIN curates information about genetic perturbations, gene expression, phenotype, gene function and human disease model data from zebrafish research publications and makes this data available to researchers worldwide. Over the past 20 years, zebrafish have increasingly been used to investigate the effects of chemical exposure, becoming an ideal model to study toxicity, phenotypic outcomes, and gene-chemical interactions. Despite this, database resources supporting zebrafish toxicology and environmental exposure research are limited. To fill this gap, ZFIN has begun to expand its functionality to better incorporate and convey toxicology data. ZFIN annotations for gene expression, phenotype, and human disease models include information about genotypes and experimental conditions used. One type of experimental condition the database captures is the application of chemicals to zebrafish. ZFIN annotates chemicals using the Chemical Entities of Biological Interest Ontology (ChEBI) along with the Zebrafish Experimental Conditions Ontology (ZECO) to denote route of exposure and other experimental conditions. These features allow researchers to search phenotypes and human disease models linked to chemicals more efficiently. Here we discuss how experimental conditions are displayed on ZFIN web pages, the data displayed on chemical term pages, and how to search and download data that is associated with chemical exposure experiments.

STRATEGIES FOR RECRUITING AND RETAINING ANIMAL TECHNOLOGISTS IN A POST-PANDEMIC WORLD

Mollie Millington[1]

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The Covid-19 pandemic has reshaped work expectations, with hybrid working becoming more common. However, the animal technology industry faces unique challenges in recruitment and retention due to the need for daily animal care, regardless of holidays or weekends. This presentation will look at the impact of pandemic-induced changes in expectations about work-life balance, along with intergenerational work habits, the investment of time and effort into training someone new to the animal technology field, and how these factors influence employee retention.

Training an animal technologist typically takes one year, and the role's physical demands and involvement in animal culling can deter long-term commitment. In this talk we propose strategies to improve job advertisements, manage new hire expectations, and structure a 12-month training program to develop employees into valuable team members.

Effective communication is crucial during recruitment, onboarding, and training. Clear expectations regarding the necessity of onsite work, weekend and holiday shifts, and specific duties such as manual labour and culling should be communicated both verbally and in writing. Additionally, new employees from Generation Z often desire to rapidly diversify their skill set, receive frequent positive feedback, and implement changes to work processes quickly based on their observations. Providing clear training objectives with achievable milestones can help maintain progress and manage their expectations in their development.

In scientific settings, implementing changes to daily animal husbandry and care routines requires careful consideration of potential impacts on research and animal behaviour. Managers may also need to teach emotional resilience to new hires, as culling can be distressing. Building a positive working environment where all team members work towards common goals is essential for success in developing a team that works together well and is able to provide exemplary customer service while ensuring high level of animal welfare and care.

HARMONISATION OF EDUCATION AND CAREER PATHWAYS FOR LABORATORY ANIMAL CARETAKERS, TECHNICIANS AND TECHNOLOGISTS

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In animal experimentation, good animal welfare, high quality science and a secure Culture of Care is essential. This, in turn, requires competent, confident and caring animal caretakers, technicians and technologists.
The level and quality of educational activities of this staff, hereunder basic education as well as training and continuing professional development (CPD), varies considerably, though, between European countries. This hinders harmonisation of education within Europe, which makes it difficult to structure clear career pathways and relevant CPD activities for caretakers and technicians.
The Federation of European Laboratory Animal Science Associations (FELASA) and the EFAT established a joint working group to scrutiny educational and CPD programmes as well as career pathways for laboratory animal caretakers and technicians throughout Europe, and to make recommendations for harmonization of education and career pathways for this category of staff, with the intention to help establish new, or improve existing, education, training and CPD activities.
A survey directed to educators and other relevant persons around Europe confirmed the vast diversity between countries. The working group therefore established a five-step career staircase, with levels C0-C4 based on learning outcomes for the relevant functions described in the European Commission's Education and Training Framework. The career staircase intends to provide detailed guidelines for consistent basic and continuing education and training, as well as for the competencies required for each level. The levels should be applied for creating and adapting education, training and CPD activities for animal caretakers, technicians and technologists throughout Europe.

HOW TO DEVELOP A TRAINING AND COMPETENCY PROGRAMME ENCOMPASSING AQUATIC SPECIES

Helen Bailey[1]

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Under both EU Directive 2010/63/EU and the UK equivalent - the Animals (Scientific Procedures) Act, all living vertebrates are protected, including zebrafish. But whilst it is now becoming standard to have a training and competency programme for mice and larger mammalian species, why is it that this is not the case for aquatics species? Zebrafish should be treated on a level with rodents and that means having people who are trained and competent performing both regulated and husbandry-related procedures. Within the Biological Research Facility (BRF) at the Francis Crick Institute, we have an established training and competency programme covering all the species we work with – mice, ferrets, opossums, rats, zebrafish, guppies and X.Laevis. Having a training and competency programme in place is also an advantage to ensuring robust scientific results and to aiding technician development.

This presentation will cover some of the starting points you need to consider when developing your own training and competency programme, including:

- Why a training and competency programme is needed.
- Who should be training and assessing.
- What DOPS are and how to create them.
- Recording competency
- Planning for re-assessments.

Finally, I will point you in the direction of some resources to help you get started.

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ABSTRACTS OF POSTERS

1. ESSENTIAL GUIDANCE FOR LARGE-SCALE MANAGEMENT OF THE AFRICAN TURQUOISE KILLIFISH, *NOTHOBRANCHIUS FURZERI*

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In the last few years, the African killifish *Nothobranchius furzeri* has emerged as an important model system for the study of vertebrate biology. *N. furzeri* is an annual fish that inhabits seasonal freshwater ponds in the southeast of Africa and is characterized by rapid growth and early sexual maturation. The short median lifespan of 3 and 7 months reflects an adaptation to the ephemeral nature of its habitat making this fish the shortest-lived vertebrate that can be bred in captivity. Despite its short lifespan, *N. furzeri* recapitulates typical age-dependent phenotypes and pathologies, thus representing a valuable model for aging research. The challenge of maintaining large colonies of turquoise killifish under standardized conditions holds also the need to ensure the animal welfare and data reproducibility and translatability. The aim of our work is to provide an optimized protocol for large-scale management of colony which may serve as essential guidance to the personnel involved in the husbandry of this species. Noteworthy, the daily monitoring of fish under laboratory conditions led us to set-up an optimized methodology, compliantly to killifish biology and ethogram. We based our setting on the use of commercially available materials to enhance its reproducibility across laboratories worldwide. In particular, we focused on the identification of: 1) appropriate environmental parameters in the commercially recirculating aquatic systems; 2) adequate density according to the stages of development; 3) the most suitable feeding strategies for each age. The novelty of our protocol is also represented by the detailed description of the most common procedures, *i.e.* fin clipping, intraperitoneal injections of various substances and non-lethal blood sampling along with the related optimized anaesthetic protocols.
With the definition of this protocol, we want not only to provide a valuable guidance to researchers interested in working with *Nothobranchius furzeri* but also to contribute to defining welfare guidelines specific to this new species.

3. DETERMINING THE LINK BETWEEN HORMONES, OPIODS AND TASTE PERCEPTION IN ZEBRAFISH: A PILOT PROJECT WITH IMPLICATIONS IN AQUACULTURE.

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*: These Authors share the last authorship.

Zebrafish *(Danio rerio)* is a widely used model organism in scientific research, and understanding their feeding behavior is crucial for optimizing their care and management. Previous research in mammals has shown that hypothalamic orexigenic and anorexigenic hormones play a critical role in driving food preference, with the endogenous opioid system emerging as a significant player in food intake. However, the role of these systems in fish, particularly in the context of food preference, remains largely unexplored. This study investigated whether the hormones that regulate hunger and satiety also influence food preference and selectivity in zebrafish. We conducted a series of experiments using male and female zebrafish, focusing on their feeding behavior in response to a standard diet (SD) and a high-fat diet (HFD). Naloxone, an inhibitor of the endogenous opioid system was used to mechanistically inhibit the ability to choose food, thereby assessing its impact on food preference. The SD group was habituated with the standard diet for 7 days and continued SD feeding until the end of experiments, while the HFD group started the feeding regimen with SD for 7 days and then switched to a high-fat diet. Naloxone treatment was administered to fishes from SD and HFD groups (but not controls) on the last experimental day.The results indicated that the two different diets did not lead to different growth rates between groups. However, in the HFD groups of both females and males, a significant increase in food intake was found, indicating the augmented palatability of HFD. Naloxone treatment induced a significant reduction in food intake, denoting that inhibition of the opioid system can reverse the previous findings. Gene expression analysis revealed that several orexigenic and anorexigenic genes displayed significant differential expression in SD vs. HFD and in naloxone vs. HFD groups, providing a possible explanation for the observed behavior. Orexin is upregulated in the HFD diet compared to SD, while naloxone treatment reverses this trend. On the contrary, nucleobindin2/nesfatin-1 appeared to be downregulated in HFD vs SD and upregulated with naloxone treatment. Cocaine-amphetamine regulated transcript showed downregulation in HFD vs SD but is not affected by naloxone. Intriguingly, both proopiomelanocortin and its receptor Mc4r were upregulated in HFD vs SD conditions, and naloxone treatment results in strong deregulation. These genes showed different expression trends in different tissues, indicating a tissue-specific involvement and activation of circuits driving food intake. Genes involved in taste perception also appeared to be differentially expressed, with an increase in

CD36, the “fat receptor,” in HFD groups. Taken together, the preliminary results indicate a complex, tissue-specific interplay of orexigenic and anorexigenic genes and the involvement of taste in driving preferences for highly palatable food. In addition, blocking the opioid pathway resulted in the reversal of feeding behavior, explainable through the regulation of key genes. This study provides novel insights into the hormone-opioid-taste axis and its role in food intake behavior, enhancing our knowledge of zebrafish feeding habits and supporting the development of updated feed composition recommendations for fish in laboratory settings. Additionally, these findings lay the groundwork for scalable studies in other fish species used in aquaculture.

Acknowledgements: This research was supported by the University of Saskatchewan Global Innovation Fund to SU, LDA & PdG, and a Discovery grant from the Natural Sciences and Engineering Research Council of Canada (NSERC) and Centennial Enhancement Chair in Comparative Endocrinology to SU.

4. POTENTIAL TOXICOLOGICAL EFFECTS OF FUNGICIDES MIXTURES IN ZEBRAFISH EARLY LIFE STAGE

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Folpet, penconazole and metrafenone are fungicides that act through different mechanisms, and are commonly used in agriculture, also in mixtures, to counteract the action of fungal pathogens. Their widespread use has inevitably led to the accumulation of these substances in soil and water, with potential damages to the entire ecosystem. The toxic effects of these fungicides in zebrafish are poorly investigated; thus, the aim of this study was to evaluate the acute toxicity induced by such compounds alone and in mixture. First, we investigated acute toxicity of the single compounds on zebrafish at early life stages to calculate the lethal concentrations and assess developmental alterations. Zebrafish larvae exposed to folpet
and penconazole at 96 hours post fertilizations showed LC 50 of 1.04 mg/L and 2.75 mg/L, respectively, while metrafenone did not reach the LC 50 but only the LC 10 (0.57 mg/L). Based on these data, three sub-lethal concentrations were selected, namely, 0.25 mg/L, 0.5 mg/L and 1 mg/L to prepare binary and ternary mixtures. The following endpoints were assessed: blood stasis, alterations of heartbeat, pericardial edema, alteration of the blood circulation deformation of yolk sac, yolk edema, deformed eyes, tremors, deformation
of notochord, deformed head and deformed tail. Combined treatments determined greater effects compared to individual compounds. The SynergyFinder+ algorithm, used to calculate synergy scores for binary and ternary mixtures, confirmed the high compounds' synergism, especially regarding neurological effects like head deformation and tremors.
These effects were better investigated at molecular level by analyzing the expression of a panel of genes involved in the cartilage and cranio-facial development (e.g., dlx5a, sphk1, col11a1, col2a1a) and in tremor phenotypes (e.g. gabrb2, adora2a, drd2, sncga). The single compounds differently affected these genes, with folpet being the more potent. In addition, the binary mixtures containing folpet, especially folpet+metrafenone, strongly induced the genes associated with cranio-facial development. In the third phase to support the results obtained morphometric evaluation of the cranio-facial cartilage through Alcian Blue stainless and the characterization of the behavioral phenotype confirmed this complex situation. Considering that most pesticides, herbicides, and fungicides are marketed as mixtures, these results are particularly interesting as they show how the activity of single fungicides
is potentiated by the association with other fungicides, resulting in a synergism, and thus a greater toxicity, in zebrafish at early-life stages.

8. ENGINEERED BACTERIOLYTIC ENZYMES AS AN ALTERNATIVE TO ANTIBIOTICS FOR TREATING INFECTIONS IN AQUACULTURE

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The escalating threat of antimicrobial resistance represents a pressing global health challenge necessitating immediate and coordinated intervention. Consequently, developing robust strategies to prevent, control, and treat bacterial infections while minimizing antibiotic usage is paramount. Enzybiotics (enzyme + antibiotic), naturally occurring or engineered bacteriolytic enzymes, offer a promising alternative to conventional antibiotics due to their specificity, efficacy, and reduced likelihood of resistance development. We focus on peptidoglycan hydrolases (enzymes that modify bacterial cell walls), determine their structure, specificity, and stability, and transform them into potent agents against specific pathogenic bacteria. We have developed nearly 40 enzybiotics and demonstrated their impressive capability to selectively and efficiently eliminate even more than 99% of target pathogens e.g. staphylococci, streptococci, enterococci, including antibiotic-resistant strains. In this study, we present a novel enzyme designed to fight *Yersinia ruckeri*, bacteria which by causing yersinosis becomes a major constraint to the expansion of salmonid culture worldwide, affecting both Atlantic salmon (*Salmo salar*) and rainbow trout (*Onchorhynchus mykiss*). The antibacterial properties of our enzybiotics were tested *in vitro* on different bacterial species. We confirmed its significant antimicrobial potential and high specificity by monitoring cell lysis and the number of surviving bacterial cells after the treatment. In addition to biochemical studies, toxicity assessments were performed *in vitro* on mammalian cell lines and *in vivo* on the great moth (*Galleria mellonella*) larvae and zebrafish embryos. Due to the unique nature of enzybiotics, we initially anticipated that they would be entirely safe for animals. However, it has become clear that fish are significantly more sensitive than mammalian cells and *G. mellonella*. Nevertheless, we have established safe usage conditions for enzybiotics in all laboratory models. Preliminary results from field trials with Atlantic salmon parr also confirm the safety of enzybiotics.
This work was supported by grants: NOR/POLNOR/PrevEco/0021/2019 and NOR/POLNOR/feFoodCtrl/0034/2019

11. OPTIMIZING ZEBRAFISH HUSBANDRY: ENHANCING EMBRYONIC SURVIVAL AND POPULATION HEALTH IN THE LABORATORY

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To address the challenge of low zebrafish (*Danio rerio*) survival rates during embryonic and juvenile stages, we established a comprehensive husbandry protocol to improve survival during development and maintain a healthy adult population. We divided the care process into three phases: 0–5 days post-fertilization (dpf), 6–28 dpf, and from 28 dpf until death, keeping all stages at a consistent temperature of 28.5°C. During the first 5 days, we kept embryos in Petri dishes, limiting them to 90 embryos per 30 ml of E3 medium. To minimize the transmission of pathogens from parents to offspring, we introduced an obligatory treatment of 24 hpf embryos with a 0.002% sodium hypochlorite solution. We closely monitored their development—early divisions, pharyngula stage, and development of the swim bladder—removed any abnormally developing embryos, and changed the E3 medium daily. At 5 dpf, we transferred free-swimming larvae with swim bladders to new dishes (30 larvae per dish) and fed them live rotifers (*Rotifera*) immediately, recognizing this as a critical phase for survival. From 8 dpf, we introduced dry feed (Zebrafeed >100 μm) alongside rotifers. At 14 dpf, we moved larvae to 250 ml glass beakers (15 larvae per beaker), fed them twice daily with rotifers, and gradually transitioned to feeding them 2-day hatched Artemia (*Artemia salina*) larvae while continuing with the dry feed. At 28 dpf, we transferred the fish to an automated ZebTEC system (15 fish per 3.5-liter tank), which regulated water quality and other parameters, and continued feeding them Artemia larvae along with progressively larger dry feed sizes until they reached sexual maturity. After that, we switched to feeding Hikari Micro Pellets. By focusing on regular monitoring, water quality management, and proper nutrition specifically, from 4-5 dpf with live rotifers, we successfully enhanced survival during embryogenesis and supported a thriving adult zebrafish population.

12. ADDRESSING SEX BIAS IN ZEBRAFISH POPULATIONS: A PRACTICAL ATTEMPT TO BALANCE SKEWED RATIOS

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In Zebrafish research (Danio rerio), essential outputs of animal quality control encompass metrics such as breeding performance, survival rates across different life stages, overall health status, and sex distribution. To monitor these metrics, we initiated a systematic data collection effort, examining several generations of approximately 90 zebrafish lines in our facility from 2021 to 2023.
Our comprehensive data analysis revealed significant variability in sex distribution. In extreme cases, an excess of females created challenges for experimental design and line maintenance. According to existing literature, both genetic and environmental factors—such as temperature, density, and food availability—significantly influence sex ratio distribution. It is also known that sexual dimorphism occurs during the first 30 days of zebrafish development. Consequently, we attempted to address this issue by modifying some of the environmental conditions between 5 and 28 days post-fertilization (dpf). Specifically, we adjusted both fish density and feeding schedules to increase the proportion of males. To achieve this, we conducted an experiment involving an increase in the density of fish per tank and a reduction in daily feeding frequency in certain lines, including transgenic and mutant strains, that exhibited a female-biased sex ratio.
Preliminary results indicated that the effectiveness of these interventions varied and was dependent on the genetic background of the zebrafish strains. In some cases, we observed an increase in the number of males, while in others, the modifications had no impact at all. These variations suggest that while environmental factors can influence sex ratios, the genetic background is a crucial determinant of intervention success. These results highlight the complexity of managing sex ratios in zebrafish populations and underscore the importance of studying the effects of different genetic backgrounds on both transgenic and mutant strains, as well as on wild type lines. Additionally, understanding the biological diversity within these strains is crucial for developing effective strategies for sex ratio management. To gain a deeper understanding, further data analysis is necessary, and additional research is needed to refine these strategies and explore other methods for controlling sex ratios in zebrafish populations.

14. BEHAVIORAL ANALYSIS OF ZEBRAFISH (DANIO RERIO) LARVAE EXPOSED TO ACESULFAM POTASSIUM AND FLUFENACET

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Zebrafish embryos and larvae are frequently used as models in behavioral studies providing opportunities for high-throughput screening. Changes in locomotor behavior and response to different applied stimuli can serve as sensitive endpoints for evaluating the toxicity of xenobiotics at environmentally relevant concentrations.
In this study, we investigated the behavioral effects of embryonic exposure to two xenobiotics with different applications and chemical properties: the artificial sweetener acesulfame potassium (Ace-K) and the herbicide flufenacet (FA), a member of the per- and poly-fluoroalkyl substances (PFAS).
We performed zebrafish behavioral assay using ViewPoint Zebrabox monitoring system. After embryonic exposure to low concentrations of Ace-K / FA, the 120 hpf zebrafish larvae were transferred into 96-well microplates and their locomotor activity was tracked by the system during 10 min light, 20 min dark, and 10 min light periods, measuring parameters such as distances covered in small or large movements. The activity of the larvae during the different periods of incubation and their response to light-dark transition were evaluated.
There were no drastic alterations in the motor behavior of the Ace-K / FA-exposed larvae compared to the control. However, both Ace-K and FA significantly decreased the locomotor activity during the light-periods, at the highest applied concentrations (0.2 mg/L and 1.6 mg/L, respectively). In addition, a slight decrease in response to light-dark transition and overall activity during the first half of the dark period could be detected in FA-exposed groups.
Taken together, our results demonstrated that embryonic exposure to low concentrations of Ace-K and FA induce minor alterations in the motor behavior of zebrafish larvae. Further studies are needed to explore more detailed behavioral e.g., non-associative learning-modulating effects of these compounds by combining different types of stimuli (visual, tactile, acoustic).

Acknowledgements: This work has been supported by the Ministry of Innovation and Technology within the framework of the Thematic Excellence Programme 2021, National Defense and Security sub-programme (TKP2021-NVA-22); the EKÖP-24 (EKÖP-24-VI/MATE-18) University Excellence Scholarship Program of the Ministry for Culture and Innovation from the source of the National Research, Development and Innovation Fund; and the Enviroscience Ltd. (Hungary).

15. AUTOMATED IMAGING ROBOT - A NEW INNOVATIVE TECHNOLOGY FOR THE AUTOMATED PHENOTYPING OF ZEBRAFISH EMBRYOS

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The Automated Imaging Robot (AIR) uses a deep learning-based technology for handling, dispensing, and imaging of zebrafish embryos, or other small aquatic organisms. A source plate containing the embryos to be imaged (1,6,12,24,48,96 well plate) is placed into a receptacle as well as a destination plate of choice for dispensing the photographed individuals. During the programming of the robot, one or more orientations (e.g., dorsal, ventral, lateral) and regions of interest can be selected. Then the whole plate, or one or more samples or wells are selected, as well as the modes of imaging. There are two cameras implemented within the robot for brightfield imaging, one telecentric with a liquid lens, and one microscope camera that records a pre-set number of stacks, then compresses them to obtain the highest quality of images. In addition to brightfield imaging, an epi-fluorescent imaging module can also be added in a perpendicular arrangement. High-magnification objectives with high-NA water immersion are used to obtain the highest resolution, brightness, and imaging speed possible. Tomographic imaging is also possible, within a region of interest, multiple rotations can be selected for automated imaging. This can overcome the limitations of background luminescence in epi-fluorescent microscopy for some applications or offer possibilities for 3D reconstruction of certain areas or organs. A short video of the embryos can also be recorded for the subsequent analysis of the cardiovascular system and heart rates. After programming the imaging parameters, using a single button, all selected samples will be imaged as fast as possible. The robot contains a high-speed delta robotic head, in which a replaceable polycarbonate tube is inserted. The head picks up the embryos one-by-one into the tube, adds an amount of specific fixation gel, then lowers the tube into a glass cuvette filled with distilled water, where the imaging takes place. After the imaging the embryos can be dispensed back into their original well, or into the destination plate. The images are stored on the built-in hard drive, and can be transferred by using a thumb drive, or connecting the robot to the workplace network. In this poster we would like to show the operation and possible uses of the Automated Imaging Robot.

16. UTILIZATION OF AFLATOXIN B1 CONTAMINATED CORN BY *TENEBRIO MOLITOR* AND ECOTOXICOLOGY TEST OF BYPRODUCT FRASS BY *DANIO RERIO* EMBRYO TOXICITY TESTS

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In our study our aim was to verify a method that can turn back mycotoxin contaminated crops into the circular economy. Thus the possibility of utilizing aflatoxin B1 (AfB1) contaminated corn by mealworms for later use in aquaculture were investigated and the ecotoxicology effect of the byproduct frass was studied by *Danio rerio* embryos. In the first stage of this study several corn feed samples with different concentrations of AfB1 were fed to the insects. Then analytical measurements were done with the residual to study the possibility of legal application of it and the end product insect meal. The byproduct of larvae rearing is the mixture of unconsumed feed and the larval excrement which called as frass. After 5 weeks feeding period, we found that insect mortality hasn't been increased compared to the negative control group. The highest applied concentration in the contaminated corn feed was 415 µg/kg of AfB1 (threshold for AfB1 for feed and feedstuffs is 20 µg/kg set by European Food Safety Authority, EFSA). Analyzing the leftover frass, AfB1 content was 157,6 µg/kg in case of the same highest concentration of feed sample (C4). AfB1 concentration of C1, C2, C3 frass samples were 5,1 µg/kg, 8 µg/kg, 17,3 µg/kg respectively, for CC frass sample the concentration was under the detection limit of 1 µg/kg. The frass itself deriving from the first stage of this study was further investigated from an ecotoxicological standpoint. For that the water diluted frass samples from each prior feed samples and dilution of clean corn feed were injected into *Danio rerio* embryos. Mortality of clean corn feed and the toxin free frass samples were 8,33% and 13,33% meaning that the frass itself show low toxicity. As the analytic toxin content of frass samples increase so does the mortality experienced. Frass samples caused the following mortality in the embryos C1 23,33%, C2 50,83%, C3 43,33%, C4 91,66%. However, the gradual increase in mortality percentages assume that this method is adequate to measure the toxicity of the frass samples, we are planning to work out a method with the use of dissolvent as a next step in the toxicity study. Based on our findings further investigation of this topic is more than suggested as the insect meal originated from this method utilizing AfB1 contaminated corn is assumed to be legally usable as feed ingredient. However, the toxicity of the remaining frass from treated groups is clearly higher than control, which still raising issues about the manipulation and the necessary treatment of this byproduct.

Acknowledgments:

This research was financed by the Research Excellence Programme (Z.C.-B.) and the Flagship Research Groups Programme (SZ.I., U.B.) of the Hungarian University of Agriculture and Life Sciences (MATE).

17. OPTIMIZING ZEBRAFISH SPACE MANAGEMENT: THE ROLE OF SPERM FREEZING AND IN VITRO FERTILIZATION

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The Zebrafish Core Facility (ZCF) of the International Institute of Molecular and Cell Biology in Warsaw is the largest zebrafish breeding facility in Poland. It was established under the European Union grant FISHMED (Fishing for medicines and their targets using zebrafish models of human diseases) in 2012. The funding enabled the establishment of a professional research unit, equipped with an automated professional fish breeding system, which in its current form is capable of housing nearly 27,000 adult fishes, both wild type and genetically modified lines. The unit is additionally supported with high-end equipment, including a system for behavioral studies, a state-of-the-art Zeiss fluorescence microscope Lightsheet.Z1 and microinjectors. The aim of fostering cooperation, exchange of ideas and experience, and promotion of the zebrafish model in Poland guided the establishment and operations of an innovative zebrafish scientific platform.
The daily operations of the ZCF required an efficient management system and the introduction of innovative techniques to efficiently manage the space operating within the ZCF. An example of our successful project was the introduction of the technique of sperm freezing (SF) and in vitro fertilization (IVF) into routine practice. Maintenance of all the fish lines that have been used throughout facility's history occupied a considerable space on the breeding racks, which causes a number of consequences: it contradicts the 3Rs principle which recommends the breeding of the smallest possible number of animals. The constant maintenance of all fish lines is also uneconomical, generating costs for the laboratory and requiring handling by qualified personnel who could be delegated to other tasks. SF and IVF, as techniques for securing and subsequently reproducing material, make it possible to eliminate, or significantly reduce, these negative factors. Thanks to SF and IVF, we are able to improve our management of genetic and rare fish lines as the way to preserve unique genetic material and maintain genetic diversity, with a possible backup in case of genetic drift or loss of certain strains. It also improved the long term maintenance of wild-type lines, reducing the risk of negative effects of inbreeding. Lastly, SF and IVF improve cost efficiency and space optimization, where we are able to lower costs associated with housing, feeding, and operating on large zebrafish colonies by managing the limited space of the fish facility more efficiently. We select only healthy, appropriately-aged males for SF to maintain high service quality. Sperm from males is obtained intravitally. We maintain dedicated population of females from wild-type lines exclusively for use in IVF procedures.

Our space management strategy extends beyond sperm freezing (SF) and in vitro fertilization (IVF). We have also implemented comprehensive protocols, including: limiting the age of fish to 20 months, setting an upper age limit for breeding to sustain genetic lines, restricting breeding to fish no older than the F3 generation, maintaining rigorous quality and purity control of wild-type lines, and conducting regular health examination of the fish.

18. INTRODUCTION OF A STEP-BY-STEP PROTOCOL FOR THE ERADICATION OF MYCOBACTERIUM HAEMOPHILUM IN ZEBRAFISH SYSTEM

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In 2017, the zebrafish unit at University of Glasgow experienced a detrimental outbreak of pathogenic bacterium, *Mycobacterium haemophilum*. The presence of other bacterial species was also confirmed by bacteriology growth. The affected individuals composed of a wild-origin parental population sourced from India and their F1 offspring generation (in house). Bacteria were diagnostically confirmed to be present systemically in fish and within the water and biofilm of the recirculating zebrafish systems. In the absence of a publicly accessible step-by-step disinfectant protocol for these difficult-to-eliminate pathogens, we devised a successful procedure to eradicate *M. haemophilum* and Aeromonas species after colony removal using chlorine tablets (active ingredient Sodium dichloroisocyanurate) and Virkon Aquatic. Postdisinfection diagnostics did not detect pathogens in the system or in the new fish inhabiting the system that were tested. Newly established fish colonies have not shown similar clinical signs or disease-induced mortality in the 1-year period following system disinfection and repopulation. Our aim is to provide a detailed disinfection procedure for the effective elimination of *M. haemophilum* and *A. hydrophila* from research-standard zebrafish units.
The simplicity of this disinfection protocol allows for simple adjustment to be used in different settings, such as flow-through or recirculation systems (both glass and polycarbonate designs), and can be adapted to cater to smaller or larger scales for other aquatic facilities as well. It is a cost and time effective method to use for facility or quarantine unit disinfection before the introduction of new colonies of fish.

19. DETECTION OF MICROPLASTICS IN ZEBRAFISH HOUSING SYSTEMS: CAN MICROPLASTIC BACKGROUND CONTAMINATION AFFECT THE FINAL RESULTS OF MICROPLASTIC-RELATED TOXICOLOGICAL TESTS?

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Microplastic (MP) concentrations were assessed in three commonly used zebrafish (Danio rerio) housing systems to evaluate their potential impact on laboratory microplastic-related toxicology tests. Zebrafish are extensively used as model organisms in MP toxicology studies, and many housing systems incorporate components made from various polymers. The degradation and wear of these components may release MPs into the water or fish, potentially introducing background contamination that could influence toxicological outcomes.

To accurately sample MPs from the housing systems, two in-situ filtration techniques were employed: a newly developed peristaltic pump-driven method and a jet pump-driven method. The collected MP particles were analyzed using Fourier-transform infrared (FTIR) microscopy, with a detection limit of 50 µm, and their potential sources were investigated. The peristaltic pump technique demonstrated superior MP recovery, particularly for smaller particle sizes. The most frequently detected polymers were polyester, polyethylene, and polypropylene, with the highest concentration observed at 0.31±0.12 particles/liter (0.22±0.16 µg/liter). These concentrations are negligible compared to the much higher MP levels commonly used in toxicology studies (10^4 - 2.21×10^8 particles/liter). Additionally, some detected MPs were identified as external contaminants, unrelated to the housing systems.

These findings suggest that while MPs are present in zebrafish housing systems, their concentrations are unlikely to significantly influence toxicology test results under typical experimental conditions.

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Responsible publisher:
Dr. István Szabó,
Hungarian University of Agriculture
and Life Sciences (MATE)

Editors:
Anita Rácz
Claire Allen, PhD
Zsolt Csenki-Bakos PhD
Joana Monteiro, PhD

Printing house: Vármédia Print Kft.
Made in 70 copies
Gödöllő, Hungary, 2024

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