

ZEBRAFISH HUSBANDRY ASSOCIATION: AVAILABLE RESOURCES FOR THE COMMUNITY

1ST NORDIC ZEBRAFISH & MEDAKA CARE WORKSHOP

Oslo, February 7, 2018

WHAT IS THE ZEBRAFISH HUSBANDRY ASSOCIATION?

ZHA Mission

- Promote and develop zebrafish husbandry standards through education, collaboration, and publication
- Promote professional development through social and educational functions
- Sponsor and provide educational and training programs for members and others for care, handling and research of zebrafish



HISTORY OF THE ZHA

- New England Zebrafish Husbandry Association (NEZHA) is formed (2005-2006).
- Name change to Zebrafish Husbandry Association (2007-2008).
- Became affiliate organization with World Aquaculture Society (2009).
- Became American Association for Laboratory Animal Science affiliate (2010)
- First annual meeting and peer review session at WAS (2010)
- New ZHA website, LinkedIn page, started newsletter Stripes, established webinar series (2014)
- Sponsoring events and outreach: Zebrafish Husbandry Education Online Course, Australia and New Zealand Husbandry Conference (2016)
- Provide funding opportunities for members (2017)

MEMBERSHIP BENEFITS

- Complete access to our webpage www.zhaonline.org which includes:
 - Past ZHA meetings with access to download presentations
 - List of valuable publications related to zebrafish husbandry and aquaculture
 - ZHA discussion forum
 - Our new resources section
- Registration discount to Aquaculture America
- Voting privileges for the executive board
- Access to all ZHA Webinar Series presentations and ZHA newsletters

Individual Membership

\$30.00 per year

Institutional Membership

\$150.00 per year

Vendor Membership

\$250.00 per year

VENDOR MEMBERS



HOW DO WE ACCOMPLISH OUR MISSION?

- Website www.zhaonline.org
- Webinars
- Attending Meetings/Conferences
- Working Groups
- Community Surveys
- Newsletter



WEBSITE - www.zhaonline.org



Zebrafish Husbandry Association

HOME ABOUT US MEMBERSHIP RESOURCES UPCOMING EVENTS VENDORS LINKS

Mission Statement:

The Zebrafish Husbandry Association is a non-profit organization devoted to promoting and developing zebrafish husbandry standards through:

EDUCATION - COLLABORATION - PUBLICATION

Get Involved

Zebrafish Basics

Protocol Database

Zebrafish Husbandry Publications

Research Funding Opportunities

Webinar Series

Do It Yourself Corner

Newsletter

Presentations

Surveys

Working Groups

Discussion Forum

Job Opportunities

Facility Tour Contacts



Image provided by Nick King, 2015.



News and A

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9/14/2017

Zebrafish Husbandry Association Call for S

The Zebrafish Husbandry Association is pleased to announce our annual Call for Husbandry Workshop sponsored by Aquaneering and being held in conjunction with the 2018 Zebrafish Society conference in Las Vegas, NV on February 20 – 21, 2018.

er-reviewed portion of the Annual Zebrafish Society conference in Las Vegas, NV on February 20 –

WEBINARS

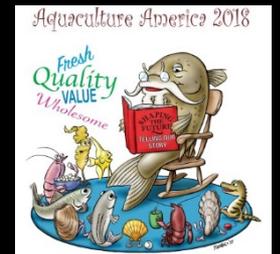
30-60 Minute Online Lectures

2017

- Special Webinar on Fish Health
 - Factors Influencing Egg Size- Dante D'India, Harvard Medical School, USA
 - Heavy Metal Contaminated Diet Affects Survival, Health, and Development of Larval Zebrafish- Marc Tye, University of Minnesota, USA
 - Fish Health and Biosecurity- Dr. Charles Innis, New England Aquarium, USA
- Zebrafish Health Screening (and Quarantine)
 - Why and How We Should Screen for Pathogens- Joanna Cambray-Young, The University of Sheffield, UK

MEETINGS AND CONFERENCES

- CALAS Symposium, Calgary, Canada
- Charles River Short Course, Foxwoods, CT, USA
- Hands-On Workshop on Advancing Zebrafish Health Programs, Lisbon, Portugal
- European Zebrafish Meeting, Budapest, Hungary
- Zebrafish Disease Models, San Diego, CA, USA
- MDI Biological Laboratory Health and Colony Management of Laboratory Fish, Bar Harbor, ME, USA
- Annual International Zebrafish Husbandry Course, Buguggiate, Italy
- AALAS, Austin, TX, USA
- Zebrafish Husbandry Workshop, Las Vegas, NV, USA
- International FishMed Conference on Zebrafish Research, Warsaw, Poland
- European Zebrafish PI Meeting, Lisbon, Portugal



WORKING GROUPS

Investigating Questions in Zebrafish Husbandry

- Larval Rearing
- Reproduction & Spawning
- Nutrition
- Water Quality
- Health Management
- Welfare and Behavior

Interested in starting a working group?
Contact Admin@zhaonline.org

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Fish Haus

The Effect of Stocking Densities on Reproductive Performance in Laboratory Zebrafish (*Danio rerio*)

Daniel Castranova,¹ Angela Lawton,^{2,a} Christian Lawrence,^{3,a} Diana P. Baumann,^{4,b} Jason Best,^{3,b} Jordi Coscolla,^{5,b} Amy Doherty,^{6,b} Juan Ramos,^{5,b} Jenna Hakkesteeg,^{7,b} Chongmin Wang,^{1,b} Carole Wilson,^{7,b} James Malley,⁸ and Brant M. Weinstein¹

Abstract

Despite the growing popularity of the zebrafish model system, the optimal husbandry conditions for this animal are not well defined. The aim of this study was to examine the effect of stocking density on reproductive performance in zebrafish. In this study, undertaken by eight different zebrafish facilities, clutches of at least 200 wild-type zebrafish embryos from a single pairwise mating were produced at each participating institution and subsequently reared according to “in-house protocols” until they were 14 weeks old. Fish were then randomly assigned into treatment groups with balanced sex ratios and densities of 3, 6, or 12 fish/L. After a 1-month acclimation period, fish were spawned in pair crosses every 2 weeks for 3 months, for a total of six spawning dates. The number of viable and nonviable embryos produced in each clutch were counted at 1 day post-fertilization. Although there was a great deal of variability in clutch size and percent spawning success among laboratories, there were no significant differences in average clutch size, spawning success, or percent viable among the treatment densities. These data suggest that using stocking densities as high as 12 fish/L does not have a negative impact on performance, when measured by reproductive performance.

Introduction

THE ZEBRAFISH HAS BECOME a well-established laboratory animal model because of its many favorable attributes, including optical clarity of the embryo, amenability to genetic manipulation, and tolerance of a wide range of environmental conditions. Interestingly, the same hardy character of the fish that has made them attractive to researchers has also delayed the optimization of husbandry conditions. Some of the most basic husbandry questions remain unanswered, including how many fish to keep in a tank, and how this affects reproductive performance. The study described in this report was organized by the Zebrafish Husbandry Association (ZHA) as a baseline study to answer these questions and provide data on clutch size, fertilization rate, and percent spawning success in different laboratories. These data have great value as a basis for the development of more formal, traditional studies¹ and

as a basic reference on reproductive performance for the fish research community.

The vast majority of research on fish densities in recirculating aquaculture systems has been on fish species raised for consumption, wherein tank densities are reported in weight/volume measurements and, in many cases, are orders of magnitude greater than current zebrafish laboratory stocking densities. The focus of much of this research is on raising fry and juvenile stage animals to the adult stage for human consumption. For example, recommended stocking densities for recirculating aquaculture systems with aeration but without direct oxygen infusion are between 30 and 40 g/L.² An average adult zebrafish weighs ~0.5 g,^{3,4} so this density recommendation converts to 60–80 zebrafish/L. Because the goals of food production aquaculture and zebrafish laboratory aquaculture are different, these numbers may not be directly relevant to zebrafish facilities. Cage stocking

COMMUNITY SURVEYS

Zebrafish Husbandry Association



Zebrafish Community Survey- The Environment (2017)

This survey is designed to anonymously poll the zebrafish community in order to capture a current snapshot of the environmental parameters in the laboratory. Thank you for participating. The results of this community survey will be made available on zhaonline.org

Stocking Density

What is your stocking density for embryos?

Your answer _____

What is your stocking density for larvae?

Your answer _____

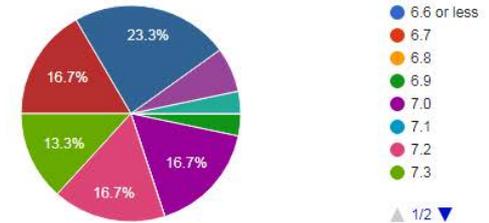
What is your stocking density for adults?

Your answer _____

What size tanks do you house your larvae?

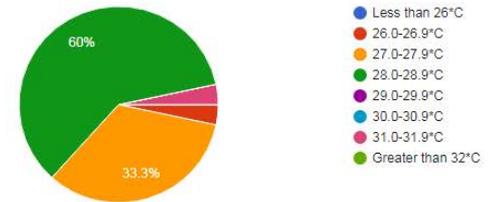
At what pH do you house your zebrafish (main breeding colony)?

30 responses



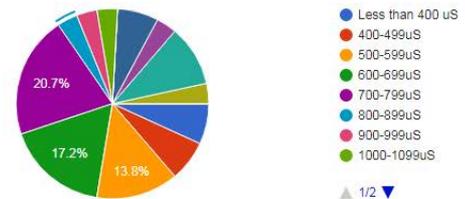
At what water temperature do you house your zebrafish (main breeding colony)?

30 responses



At what conductivity do you house your zebrafish (main breeding colony)?

29 responses



NEWSLETTERS

STRIPES

Zebrafish Husbandry Association Newsletter Spring 2017



ZHA Annual Meeting

The annual ZHA member meeting was held on Antonio Martorel River Center. The meeting was the Zebrafish Husbandry Workshop being held at a D'India and the executive board/guested members a presentation on the organization's accomplishments. They received an honorary award plaque for its executive board but an open forum session with opinions and ideas that they would like to see the topics and suggestions included a broader reach high school, new initiatives of income to help fund and set up appointments within the organization. ZHA was treated to lunch at the Gasparitas Grill.

Zebrafish Husbandry Workshop at Aquaculture
The Zebrafish Husbandry Workshop was held at Marriott River Center in San Antonio, Texas. The quality talks from presenters at across the zebrafish the two day event included Zebrafish Disease, of Review Session chaired by Dianne Crinko, and a Bobbi Bair. The keynote speaker of the event at University who gave a talk on the Evolution of the Workshop also had special presentation for JAMA as well as a special Town Hall Meeting Joint Group, IDEXX, Shering, and Ziegler which welcome CHRF to help facilitate the community discussion.

Outstanding Steward of Zebrafish Husbandry
This year's recipient of the Outstanding Steward presented to Bobbi Bair. Bobbi has been instrumental in Zebrafish Husbandry Workshop for the past eight and energy in coordinating the workshop with the living up a great round of talks year after year and strength of the zebrafish husbandry community.



President Steve Tyb presents on the accomplishments of the ZHA during the past year.

Event Spotlight—ZHA Annual Meeting at WAS 2016

The 2016 ZHA Annual Meeting was held on Tuesday Feb 23rd prior to the start of the Zebrafish Husbandry Workshop at Aquaculture America 2016 in Las Vegas, Nevada. Those who attended were greeted by the 2015 and 2016 executive boards and received a ZHA tablet and keychain. The meeting was hosted by the 2016 President Matt Tyb and the executive board for their contributions, hard work, and dedication. The meeting gave members of the community the opportunity to share the upcoming years goals for the ZHA. Members should keep a look-out in their inbox in the coming months for materials concerning future planning.

At the conclusion of the meeting, Matt presented Jim Burris with an appreciation award for his hard work and dedication as President of the ZHA in 2015 in which he commended the 2015 executive board for their contributions, hard work, and dedication.

Following the Annual Meeting, the ZHA hosted a luncheon for its members at the Terrace Garden Buffet at the Flamingo Hotel. The lunch allowed members both new and old to enjoy a casual lunch and conversation before the start of the Zebrafish Husbandry Workshop.

The Zebrafish Husbandry Workshop started in earnest with a ZHA sponsored session. A ZHA peer review board selected six talks submitted by members of the community that represented topics relating to Zebrafish husbandry. This year's talks encompassed various aspects of husbandry including acclimation, disease and pathogens, animal welfare, breeding, water chemistry, and nutrition.

Outstar Steward

The award of the Outstanding Steward presented to Bobbi Bair. Bobbi has been instrumental in Zebrafish Husbandry Workshop for the past eight and energy in coordinating the workshop with the living up a great round of talks year after year and strength of the zebrafish husbandry community.



Dr. High (member ID) providing hands on teaching.

Vendor Spotlight



The 6th International Zebrafish Course is Approaching Quickly!

Scholarships are available for qualified participants. Contact the organizer The International Zebrafish Husbandry Course will be held in Italy from Oct Center and is limited to 35 participants. Some level of scholarship is awarded based.

The international course is presented by facility managers who are active zebrafish community. Participants will gain firsthand knowledge from specialist breeding, fly growth, and management.

Find out what participants are saying about it.

Since when the Zebrafish Husbandry Course was first implemented in 2013 reputation among the Zebrafish community: Here is what participants are saying:

"This course is by far the most advanced Zebrafish Husbandry course I've ever attended. I believe whether you are an animal technician, who is just new to the industry, or you are a facility manager, or you are the most experienced researcher, you will come away from this course learning something new."

—Carl Bracker, Senior Aquarist Technician at Lancaster University.



"I didn't find it a experience... It management to an have not experience topics in such a"

—Carrie Barton, 20

Featured Facility—The Champalimaud Fish Platform Operations and Water Systems

By Ana Catarina Carita, Champalimaud Research, Champalimaud Centre for the Unknown, 1400-038 Lisbon

ana.carita@research.f Champalimaud.org

The CCU Fish Platform

The Champalimaud Centre for the Unknown (CCU) houses an ecology, ethics, and two research programs: the Champalimaud Program and the very recent Champalimaud Cancer Program.

The CCU Fish Platform initiated its operations in October 2015. It covers the fish facility which houses, breeds and maintains used for research. The Platform also provides supporting services such as breeding, live maintenance, genetic management, disease management and continuous development of husbandry, welfare and technical protocols. The semi-automated services in part of the health management program as it guarantees proper and controlled implementation of operating procedures. In 2016, the Platform has also started offering advanced services to the external scientific user generation of transgenic lines.

The Fish Platform currently serves 3 neuroscience research labs and 1 cancer research lab with a total of 24 users. The completed of a manager, 2 senior technicians and one technician, all with formal education on biology or fish biology.



Figure 1 - The CCU Fish Platform team. From left to right: Carolina Carita (manager), Tatiana Carita (senior technician), Sandra Martins (senior fish technician) and Isaura Monteiro (senior fish technician).

The Fish Facility is comprised of a main holding room with a current capacity to house more than 50,000 fish and the potential to expand to a housing capacity of 80,000 fish. It is currently fitted with 24 tanks (see supported by two independent Controlled Life Support (CLS) recirculation systems with UV disinfection (Fig. 2). The facility also holds an isolated and fully independent quarantine room with two small alone recirculation systems holding up to 2,000 fish (Fig. 3), a prax room where two 400,000 guarantee UV disinfection room-neonals (20) water production for all fish rooms (Fig. 4), and an experimental room with 3 stereoscopes, 3 microprojectors and one fluorescence microscope. All rooms are ventilated through a centralized HVAC system and are kept at controlled room temperature (22°C), 50-80% humidity. Fish holding rooms are kept under a 24/10 hour light-dark cycle with a light intensity of 200-300 lux at the water surface.

Vendor Members



Contact Us

Visit our website and media pages to learn more

admin@zhaonline.com

www.zhaonline.org

Visit us on the web at

www.zhaonline.org

If you are interested in becoming a ZHA member, vendor, or institutional member, please

contact us at

zhaonline@org and click on the Membership tab for membership pricing details and directions on how to join.

The ZHA Executive Board would like to thank all the members who contributed content and assisted with the newsletter. If you have ideas or topics you would like to see in the next edition of Stripes, or would like to provide feedback, email comments to the ZHA Executive Board at

admin@zhaonline.org.

Zebrafish Husbandry Association



FUNDING OPPORTUNITIES

- Research Funding Committee
- Zebrafish Husbandry Workshop Peer Review Session Award for selected presentations
- New funding programme being prepared in 2018
- Look for other partnerships in the near future!

Zebrafish Husbandry Research Fund Request for Proposals Draft 8 Sept 2016

The 2016 ZHA Executive Board has decided to dedicate \$1000 from the FY2016 budget to go toward funding zebrafish husbandry research.

1. Funds must be used for zebrafish husbandry research, and not for education or training.
2. The principal investigator does not need to hold a doctoral-level degree but must be a current member of the ZHA.
3. Funds may be divided between multiple proposals or given to a single proposal.
4. Grant recipients must submit a final report of their findings to the [Committee](#), are expected to publish their results in a peer-reviewed journal, are required to present their work at the ZHA sponsored session of the Zebrafish Husbandry Workshop held during the World Aquaculture Society conference, and must thank ZHA for their funding.
5. Funds are not to be used for animal costs, equipment, or travel.
6. Funds are not to be used to pay overhead to institutions or salaries.
7. If the proposed research is funded by an additional grant, the committee must contact the other grant organization and determine how to split the costs.
8. Funds may not be issued until IACUC approves the use of animals and the committee receives a letter from the institution indicating that it will accept the funding.

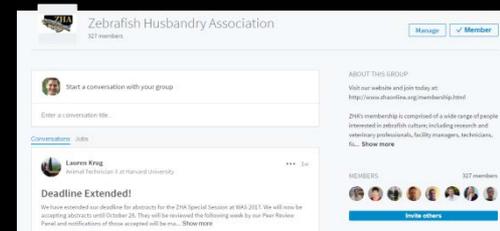
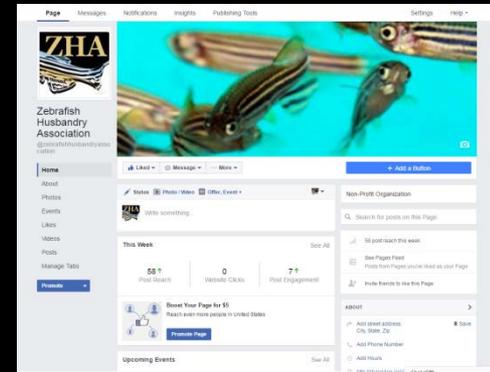
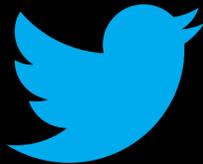
Format. Provide a 3-4 page proposal providing the following sections: 1)Background 2) Justification/Need 3) Approach 4) Budget

Literature Cited. We recommend including a Literature Cited section, and this will not be included in the page limit.

Review Process. Submit the proposals to Dr. Michael Kent, Chair of the ZHA Research Committee by 1 Dec 2016. Michael.kent@oregonstate.edu
The Committee will meet in-person at the ZHA meeting in San Antonio in Feb 2017 to discuss the grant proposals and decide which proposal(s) will be funded.

SOCIAL MEDIA

- [Facebook.com/zebrafishhusbandryassociation](https://www.facebook.com/zebrafishhusbandryassociation)
- [@ZHAonline](https://twitter.com/ZHAonline)
- www.linkedin.com



HOW CAN THE ZHA SERVE YOUR LAB?



Email - Admin@zhaonline.org



**Champalimaud
Foundation**

Impact of Dry Feeds on Zebrafish Growth and Reproductive Performance

Ana Catarina Certal, PhD

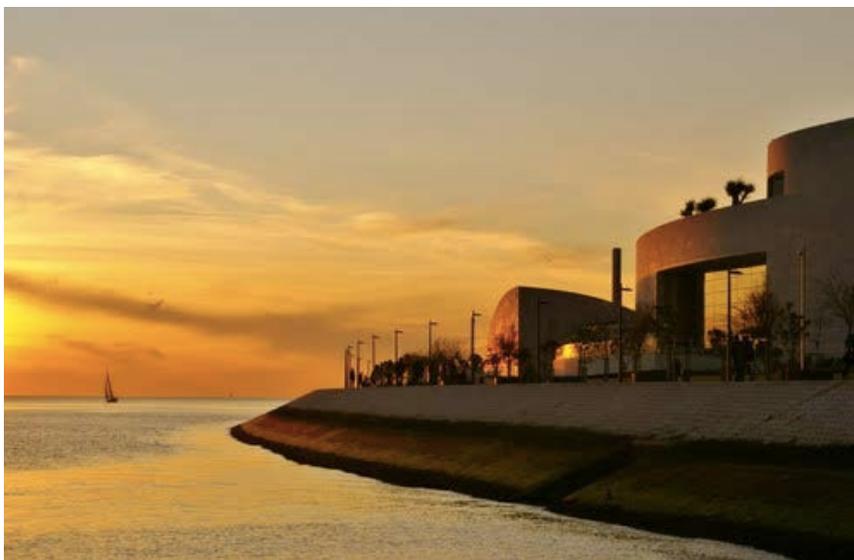
catarina.certal@fundacaochampalimaud.pt



Champalimaud Centre for the Unknown (CCU)

Neuroscience and Cancer Research

Cancer Clinical Centre





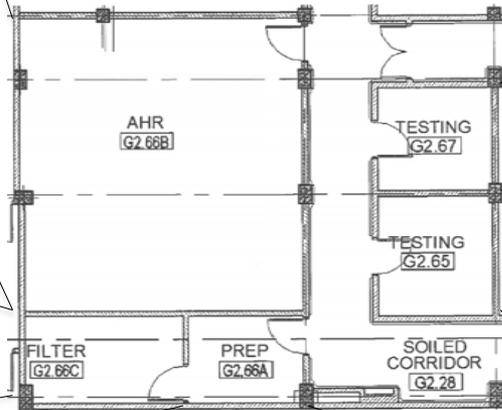
CCU Fish Platform – est. Oct 2011

Experimental room with 3 microinjection stations +
fluorescence scope

Holding room with partially automated feeding



Filter room



2-Risk level quarantine



Feed prep room

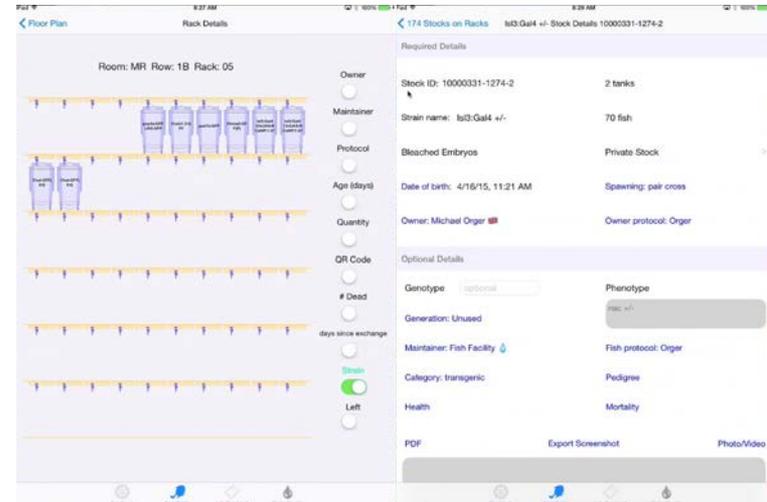


CCU Fish Platform



CORE

- ✓ Production of healthy fish for research (current capacity: ~45,000 fish; expandable up to ~70,000 fish) - CONGENTO
- ✓ Fish husbandry
- ✓ System maintenance
- ✓ Live feed production (rotifers and artemia)
- ✓ Database implementation and management (D²)
- ✓ Health program
- ✓ Embryo bleaching



ADVANCED

- ✓ Fish crosses
- ✓ Complex line maintenance
- ✓ Embryo microinjection
- ✓ Generation of new transgenic lines
- ✓ Development and coordination of genetic screens
- ✓ Advanced training & share of knowledge events – accreditation courses, Husbandry 2014, CRISPR 2015, Health 2017
- ✓ **Continuous development and implementation of new SOPs/services** – IVF, sperm cryo, genotyping
- ✓ To promote and facilitate research activities within the facility – design and set-up exp. rooms
- ✓ Development of on-demand gene editing technologies (CRISPR)





First study – choosing the best diet without live feeding as first feed



3 Commercial dry feeds:

- Gemma Micro – Skretting
- Larval AP – Ziegler
- ZM – Zebrafish Management

3 Feeding regimes:

- Dry only
- Dry + Live (Artemia + dry feed after 9 dpf)
- Control (Artemia only after 9 dpf)

M&Ms

- 3 fish strains: AB, Nacre, Tg(HuC:GCamp5G)
- 100 fish per experimental group
- 3 pellet sizes

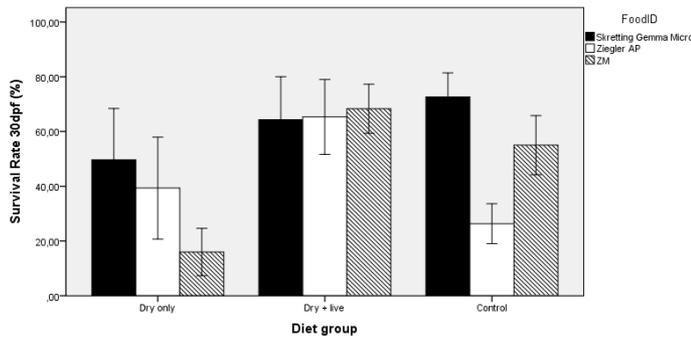
Assessment

- Survival (30 and 90 dpf)
- Growth (fork-length)
- Breeding (fecundity + embryo viability)

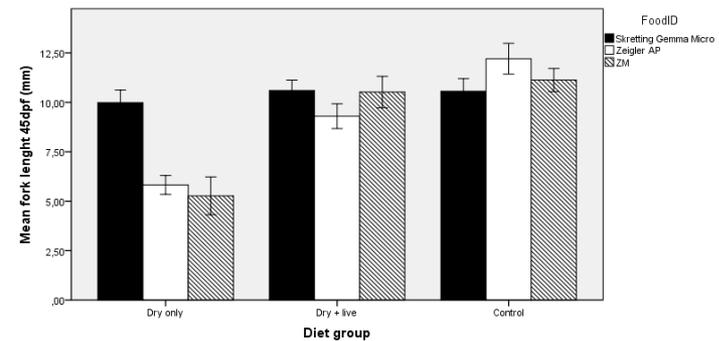


First study – choosing the best diet without live feeding as first feed

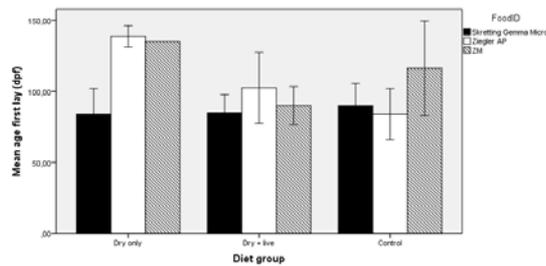
Survival



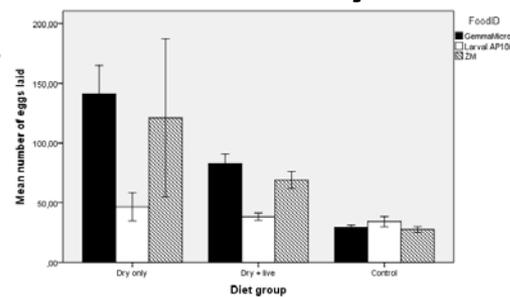
Growth



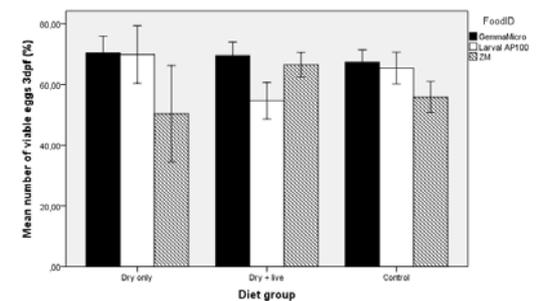
Spawning Age



Fecundity



Embryo Viability



- ✓ Gemma Micro was the only feed presenting a global alternative to live feeds during all stages – **production method – COLD EXTRUSION**
- ✓ Gemma Micro can fully substitute Artemia in later growth phases showing improved breeding features

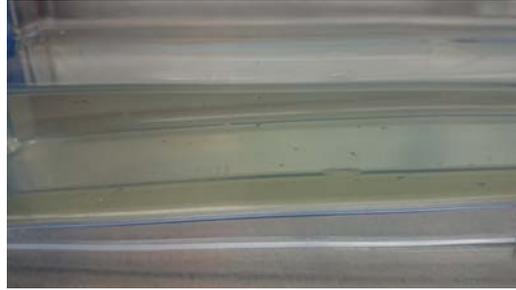


Live first feeds - Rotifers do it better



Rotifer Stock Culture

- ✓ 30% daily harvest (working days)
- ✓ No harvest at weekends/holidays
- ✓ Automated feeding



Rotifer-Larvae Polyculture



Fry-Juvenile Feeding



Rotifer O/N Feed-out Culture





Feeding Protocol

- ✓ Larvae-Rotifer Polyculture during 3 days (5-8 dpf)
- ✓ Rotifers (2x) + Gemma 150 (2x) 9-60 dpf
- ✓ Artemia (1x) + Gemma 300 (2x) 61-90 dpf
- ✓ Artemia (1x) + Gemma 500 (1x) >90 dpf



15 min

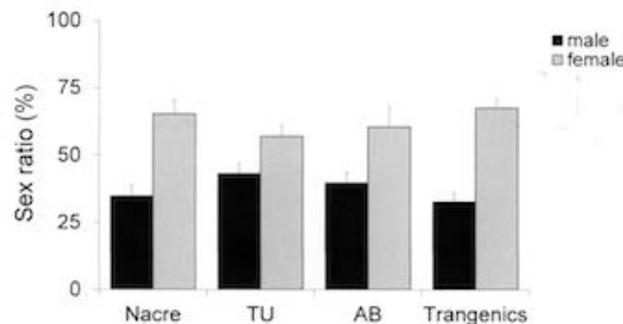
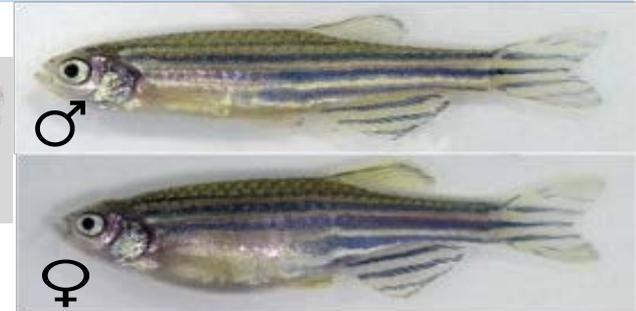
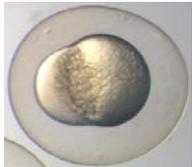
48 h

5 days

60 days @ 10 fish/L

Fertilization

CCU Fish Platform





**Champalimaud
Foundation**



congento

Lisb@20²⁰

PORTUGAL
2020



Current Staff:

Sandra Melo

Joana Monteiro

Seidy Semedo

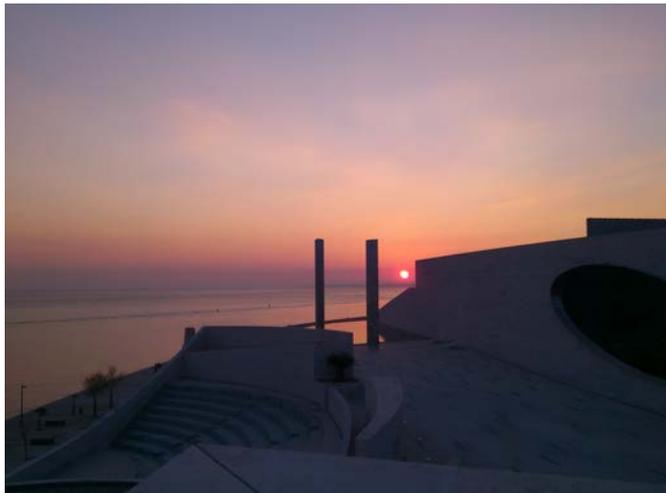
Carolina Cabrera

Rita Almeida

Former Staff:

Matheus Farias

Telma Costa



Researchers:

Michael Orger lab

Gonzalo de la Polavieja lab

Miguel Godinho-Ferreira lab

Rui Oliveira lab