A Novel Approach to Producing Reproductively Sterile Fish for Biological Containment

Ten-Tsao Wong and Yonathan Zohar

Institute of Marine and Environmental Technology

University of Maryland, Baltimore County, MD, USA

MARINE BIOTECHNOLOGY

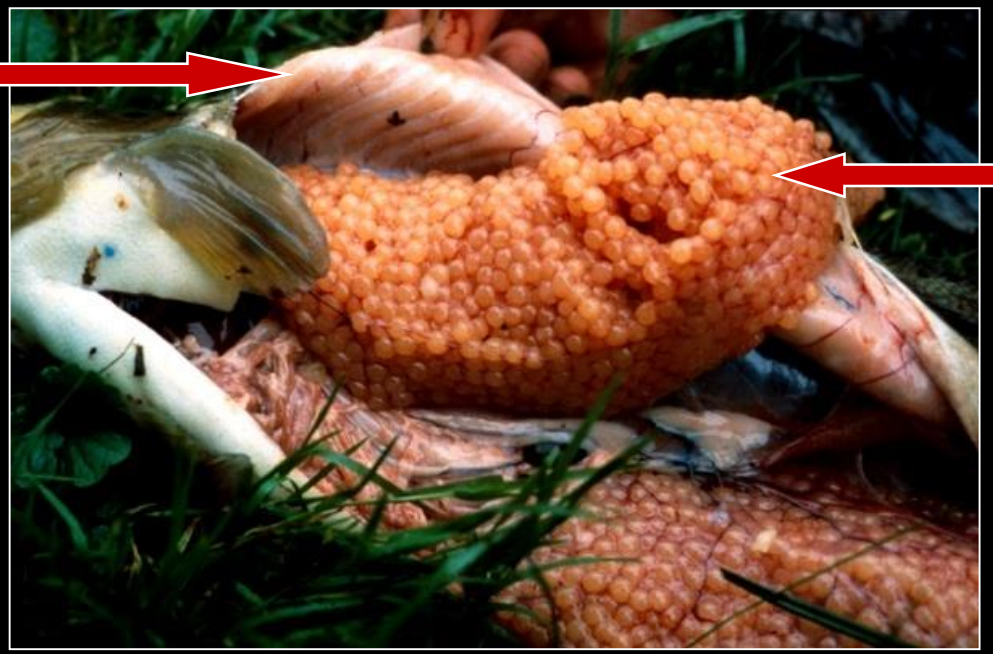
UNIVERSITY SYSTEM OF MARYLAND

UMBC

AN HONORS UNIVERSITY IN MARYLAND

Sterility of Farmed Fish: the Rationale

- Achieve better somatic growth
- Prevent deterioration of flesh quality and mortality
- Protect IP strains
- Biological containment: prevent propagation of farmed/domesticated, non-native and GM fish



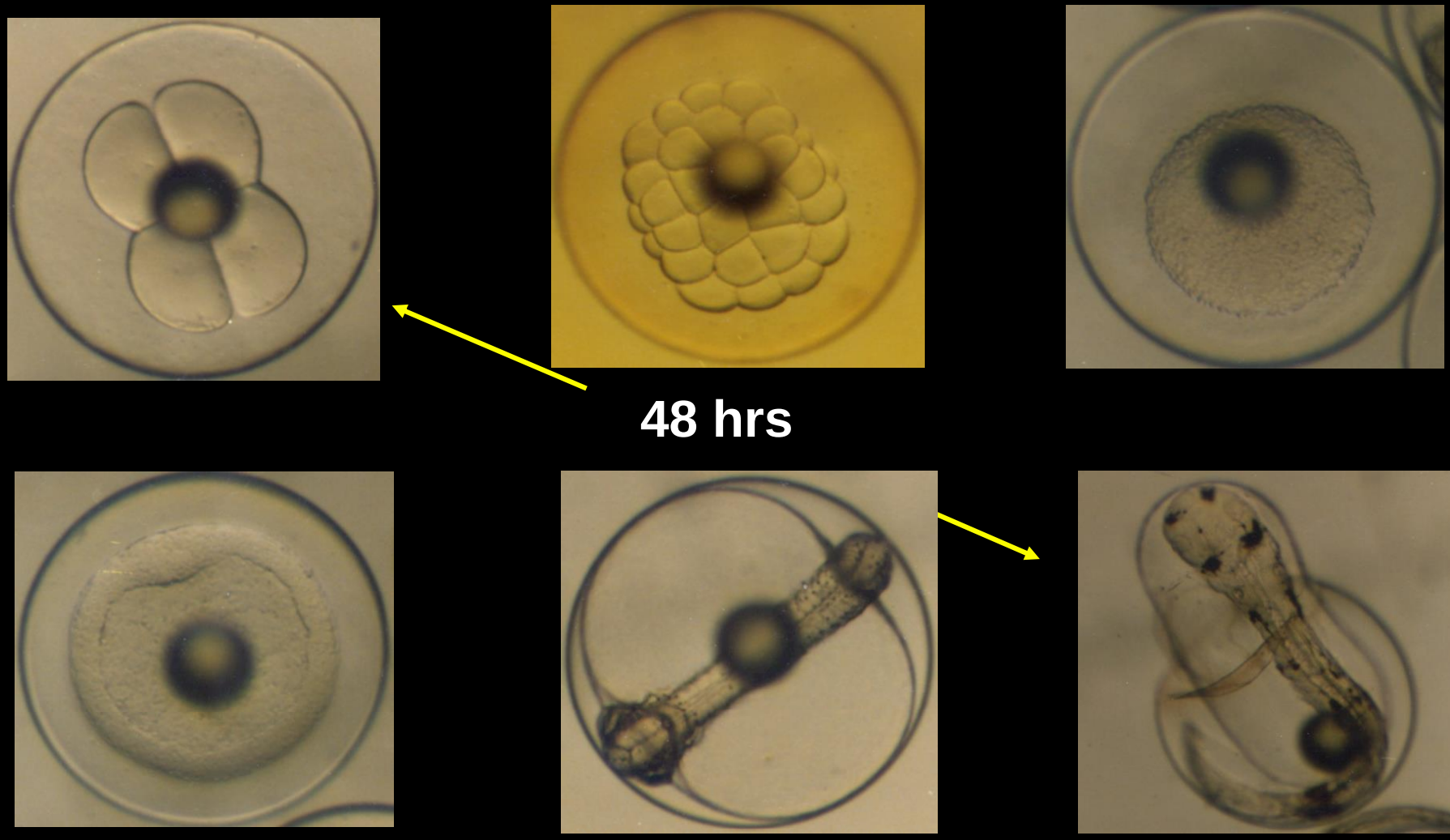
Genetic Impacts of Salmon Escapees

Farmed fish should be sterile

Recently Approved: AquAdvantage Salmon- Genetically Engineered for GH (US, Canada)

- GE Salmon must be sterile (FDA-November 2015)
- Triploid sterile salmon often display performance issues, not well received by industry

The Search for a New, Non-GMO Approach to Sterility- Disrupting Early Reproductive Development



Two Levels of Disruption Along the B-P-G Axis

-/-)

Pituitary

FSH (+) LH

Gonads

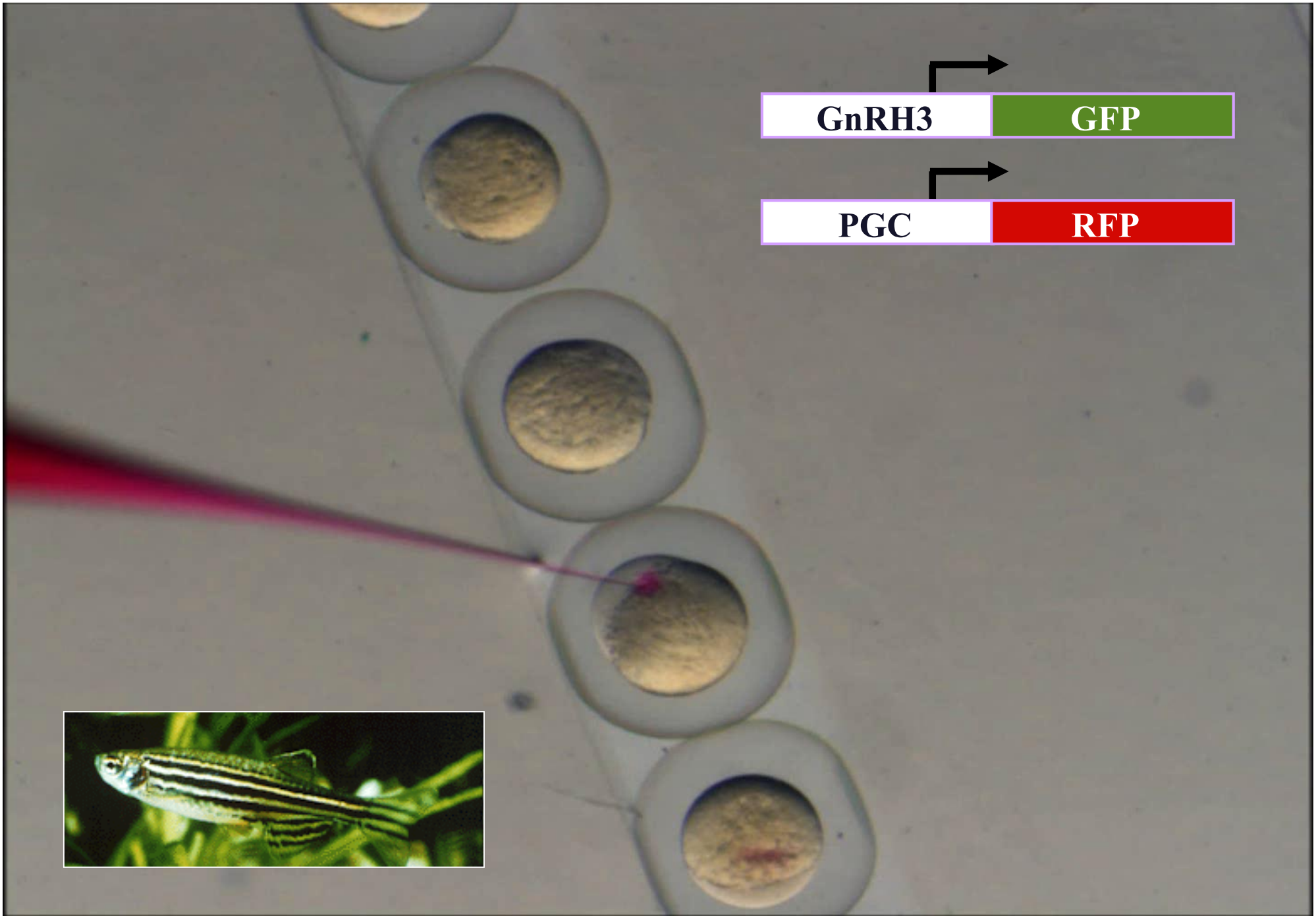
Primordial Germ Cells

Steroids

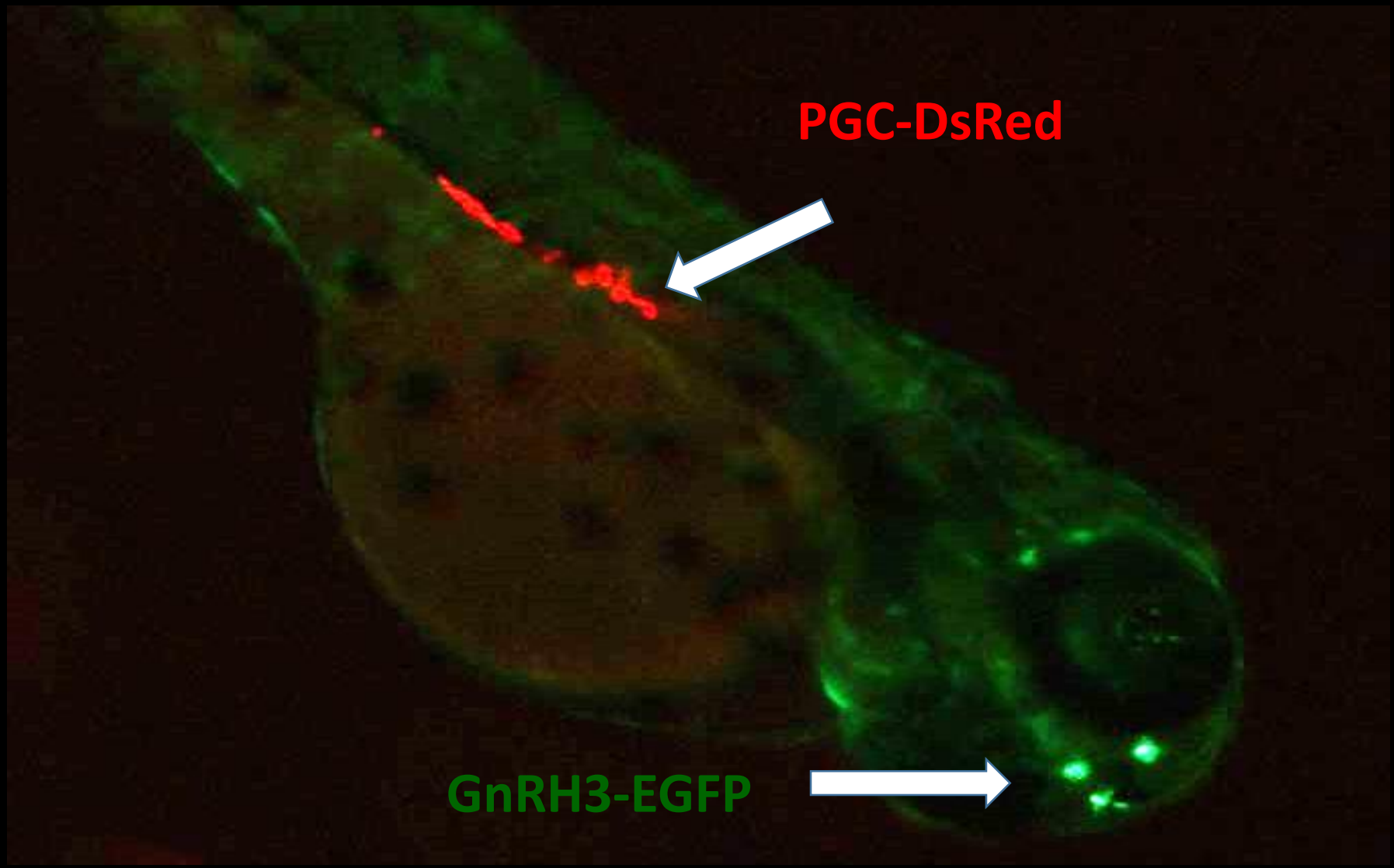
Steroids

Ovulation, spawning

Spermiation

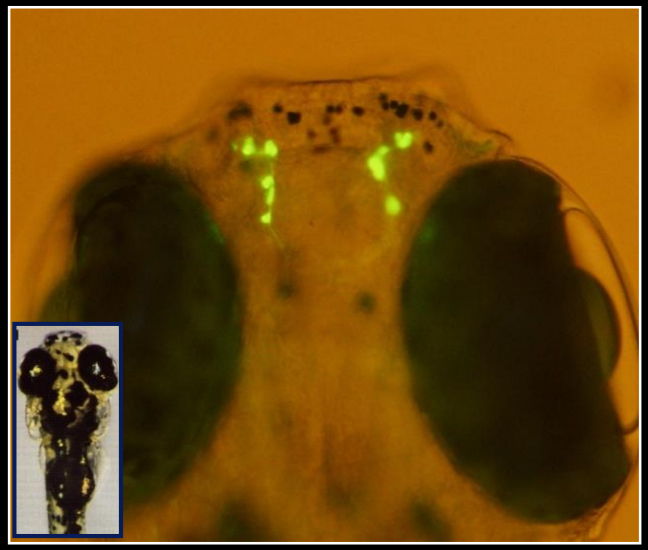

GnRH3
GFP
PGC
RFP

Tg(gnrh3:EGFP/kop:DsRed-nanos3) double transgenic fish for visualizing disruption

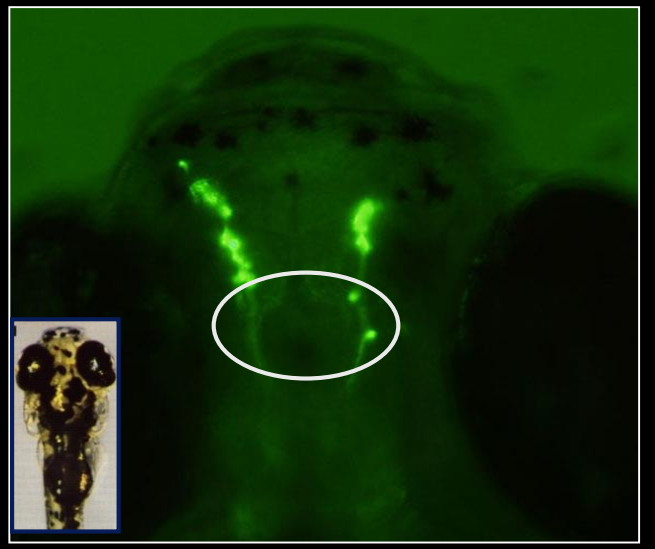


Early development of GnRH3 in Tg(GnRH3-eGFP) ZF

2 dpf



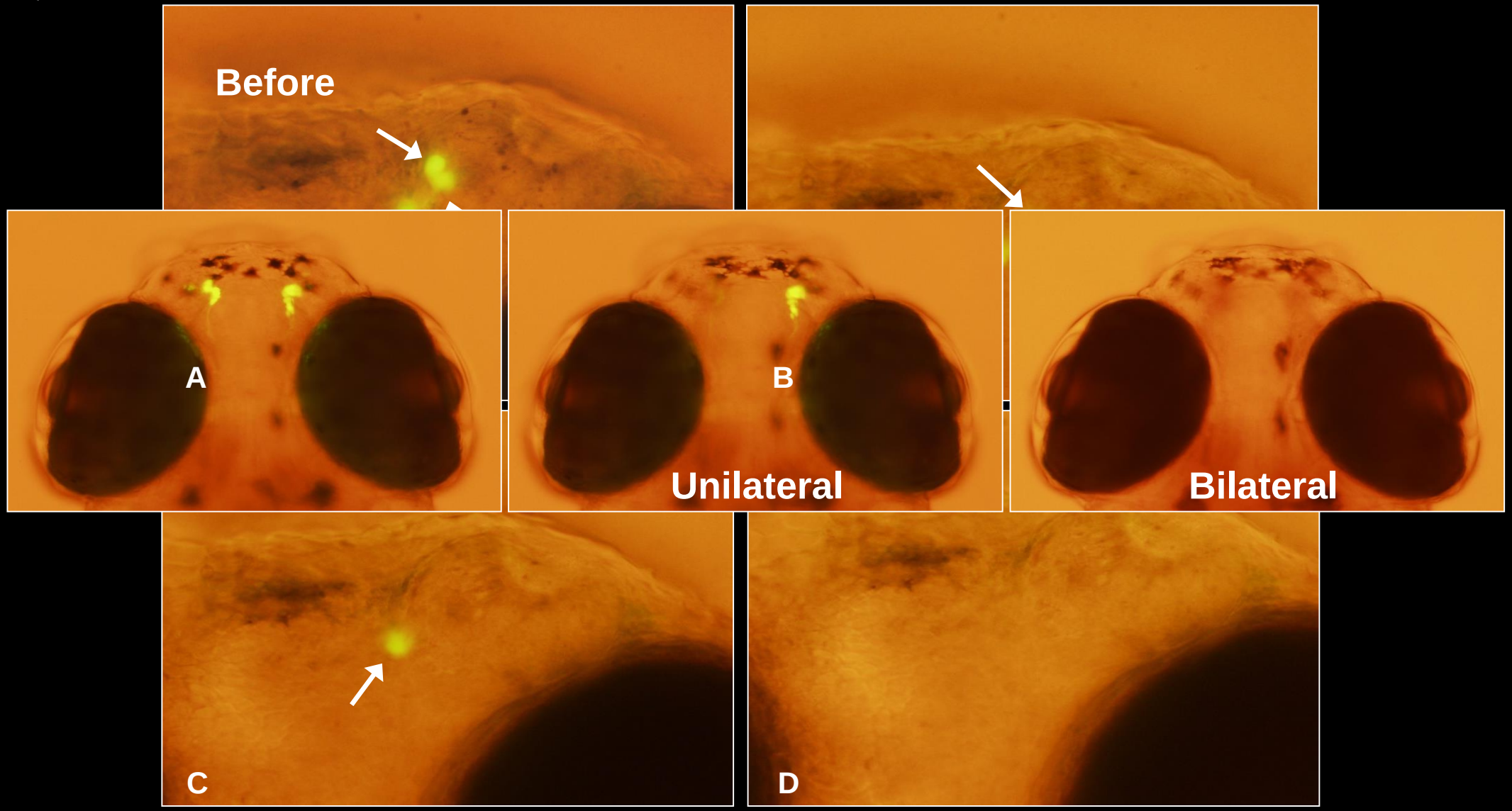
5 dpf



12 dpf

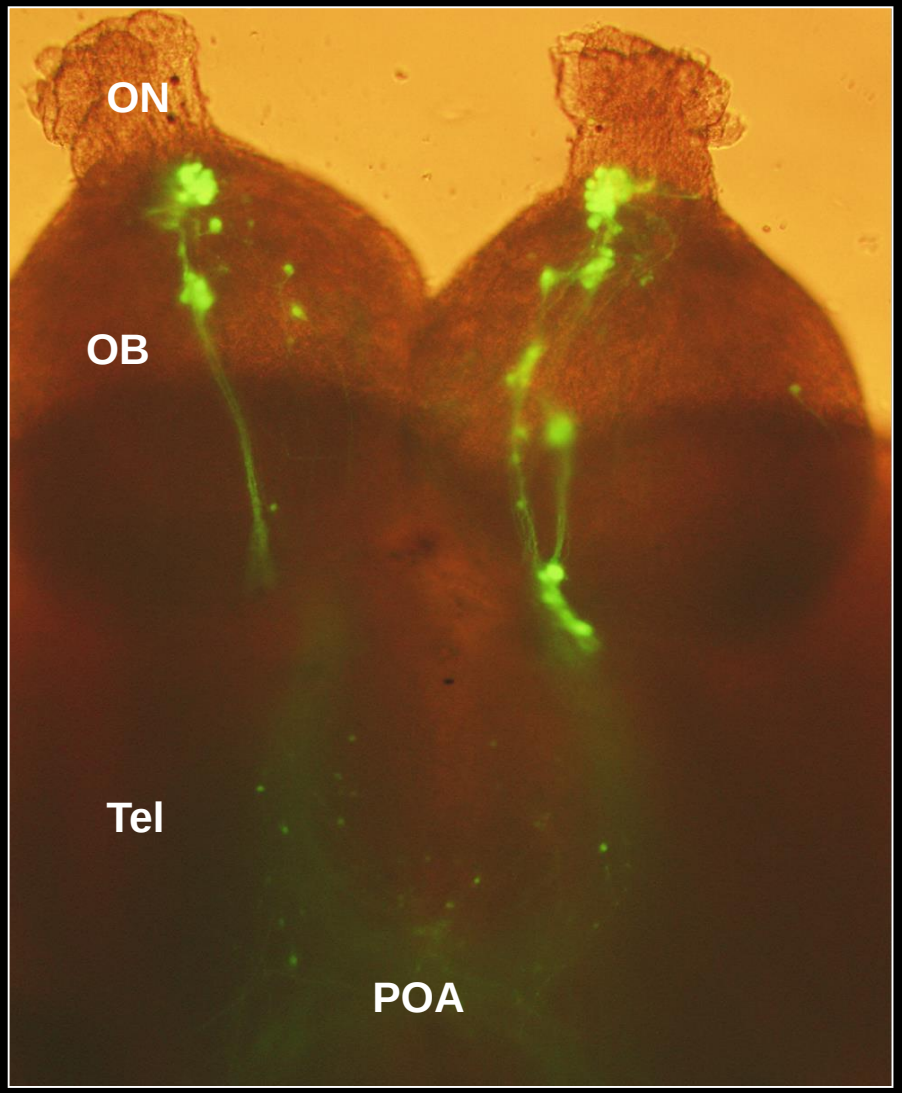
Abraham et al, 2008

Cell-by-Cell Laser Ablation of GnRH-3 Soma in the Tg(GnRH3-eGFP)



Abraham et al, 2010

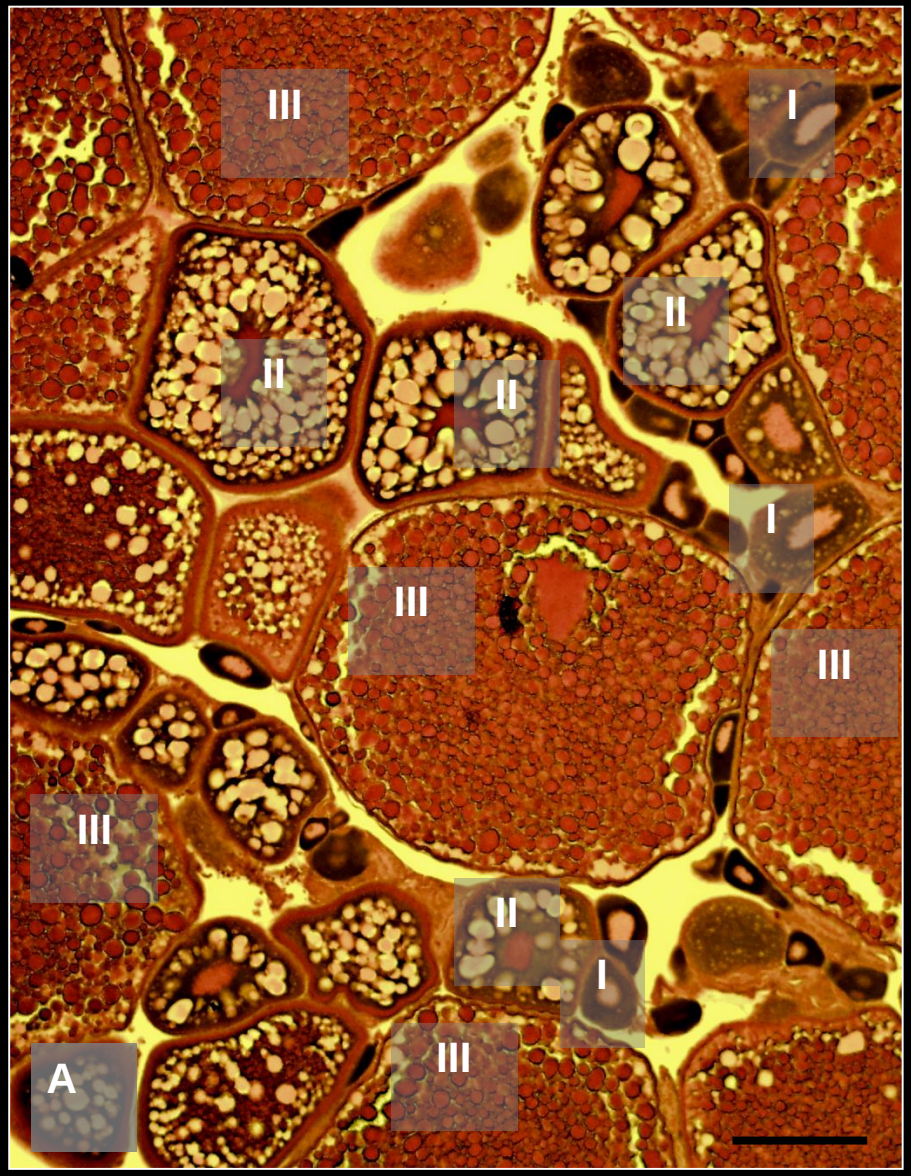
GnRH3 ablated mature CNS



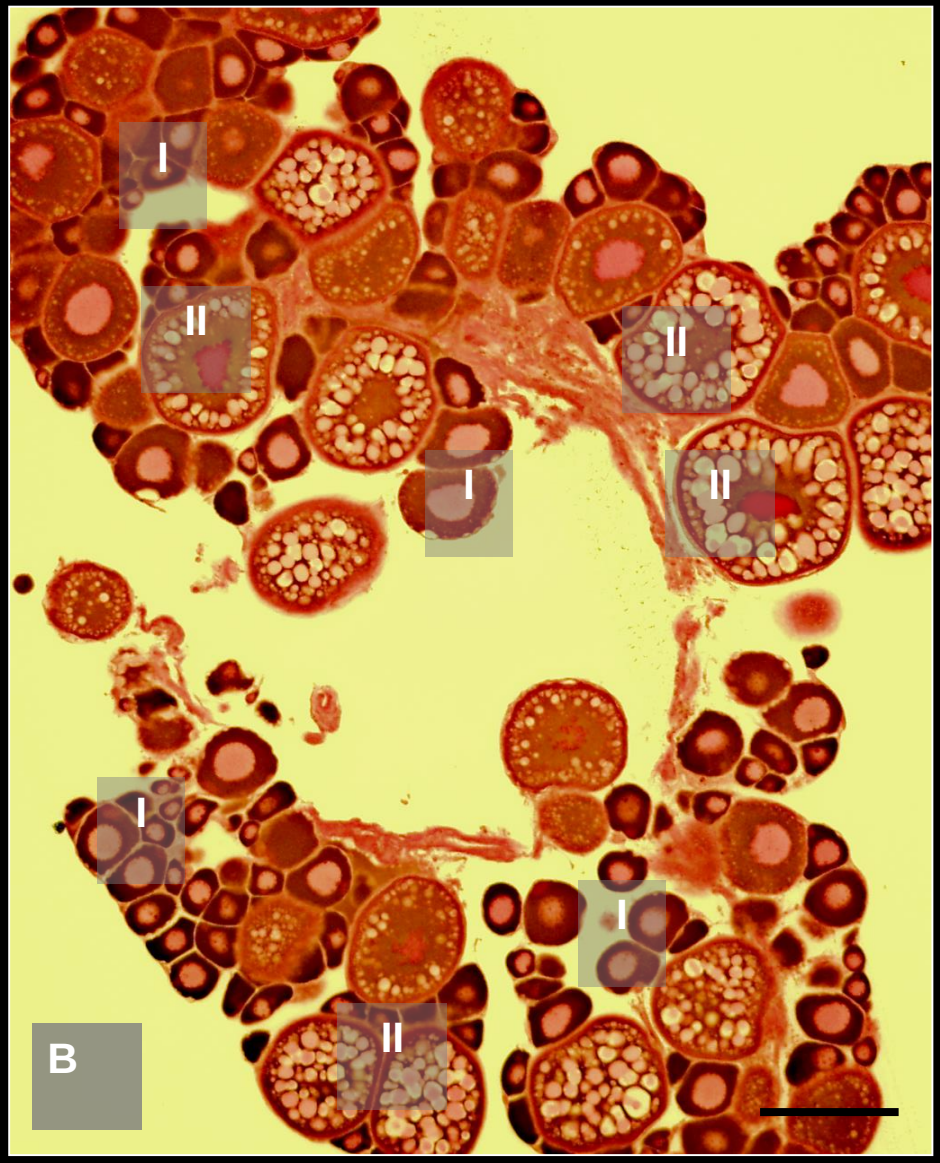
Abraham et al, 2010

Ablation of the GnRH system produces sterile fish

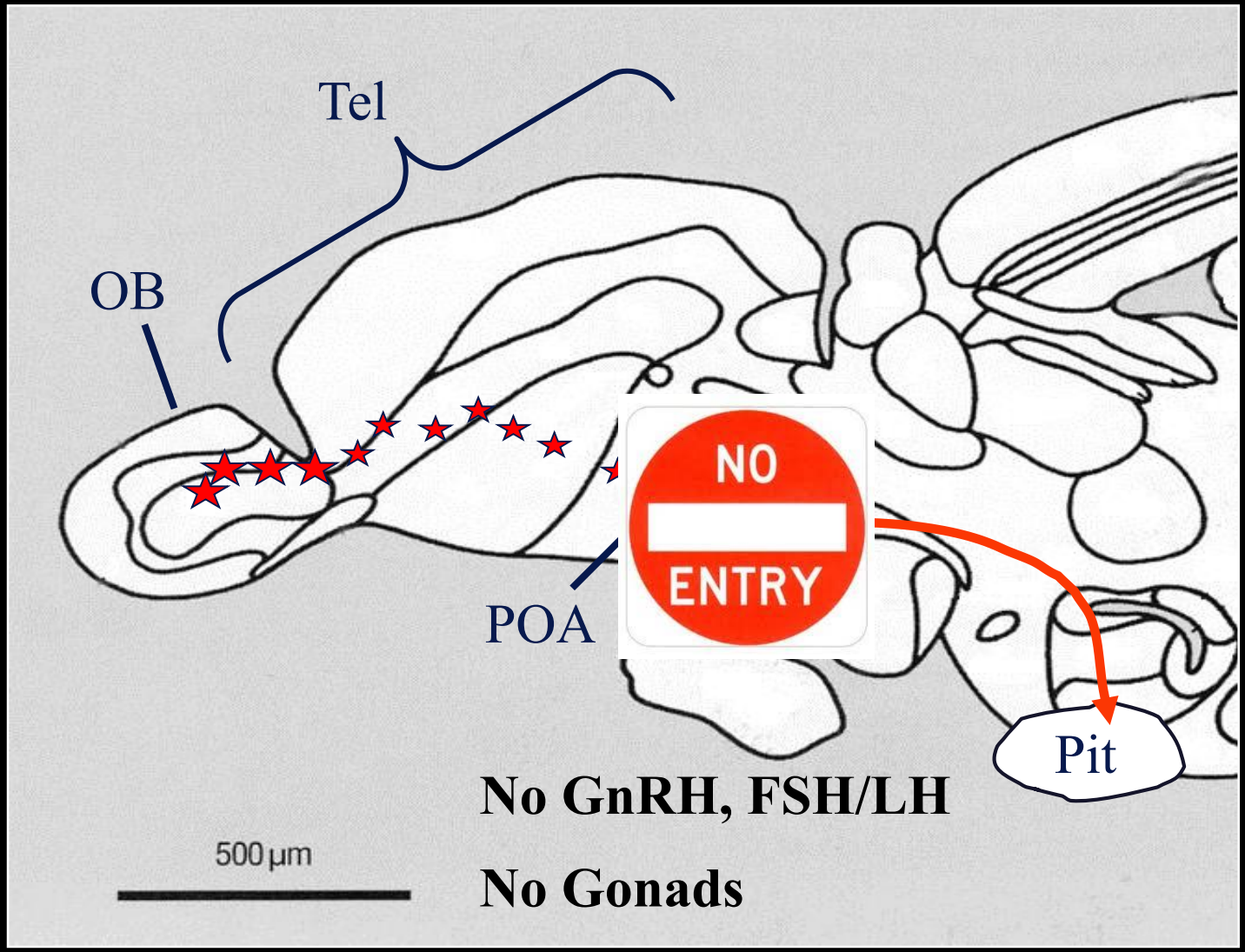
Wild type



Ablated



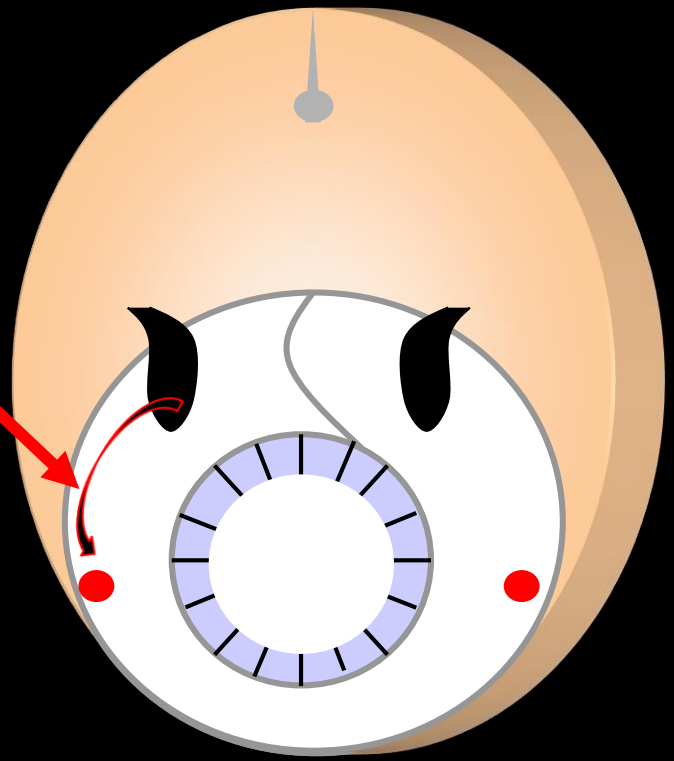
A new approach to sterility 1: disrupt the early establishment of the GnRH system



Early Migration of Primordial Germ Cells (PGCs)

Migrating primordial germ cells

Sdf-1a gradient



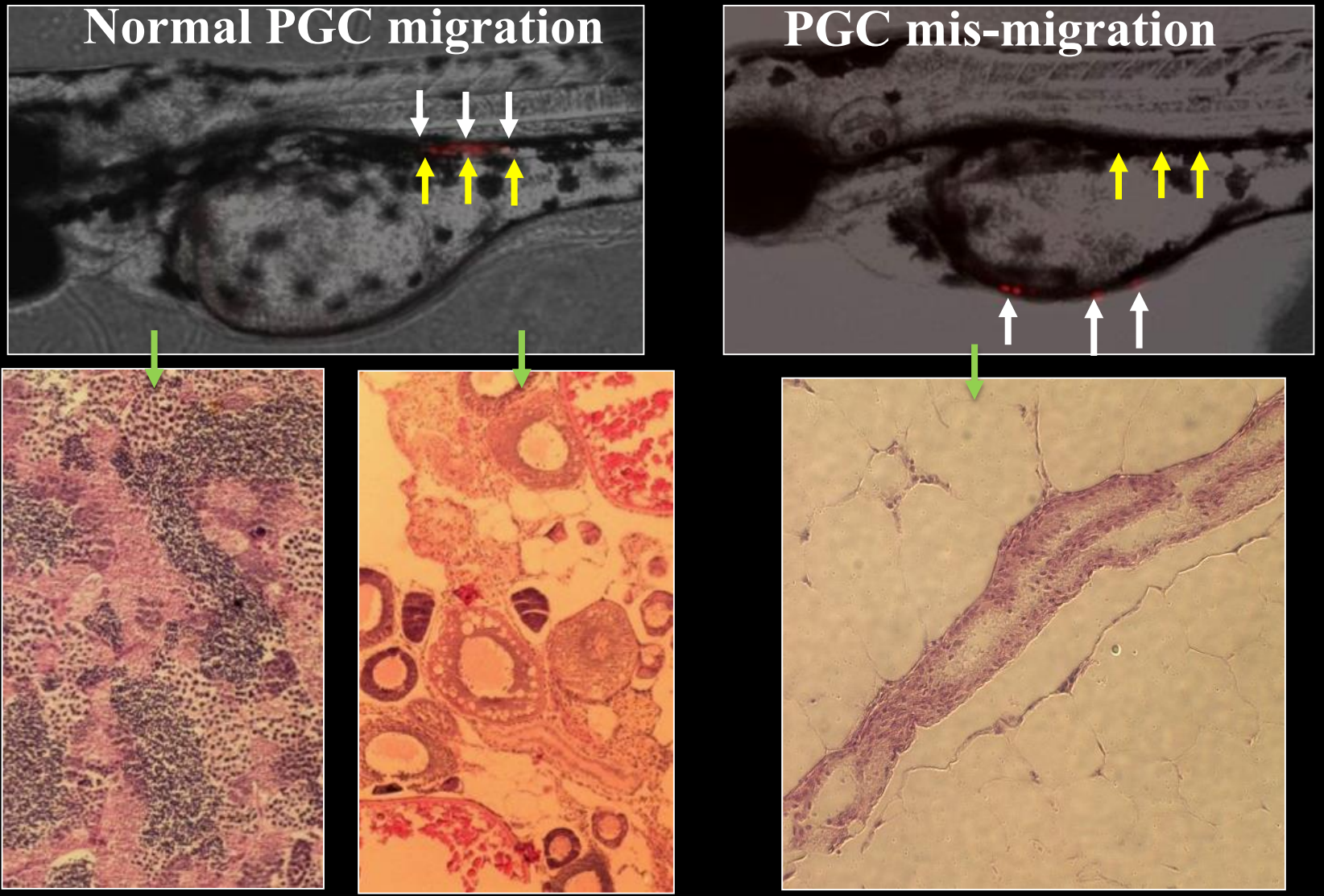
PGCs are reproductive “stem” cells that migrate to the developing gonad guided by a gradient of stromal-derived growth factor (Sdf-1a)

Modified from G. Yoshizaki

Overexpressing Sdf-1a in ZF results in mis-migration of the PGC and sterile fish

PGCs

Gonadal ridge

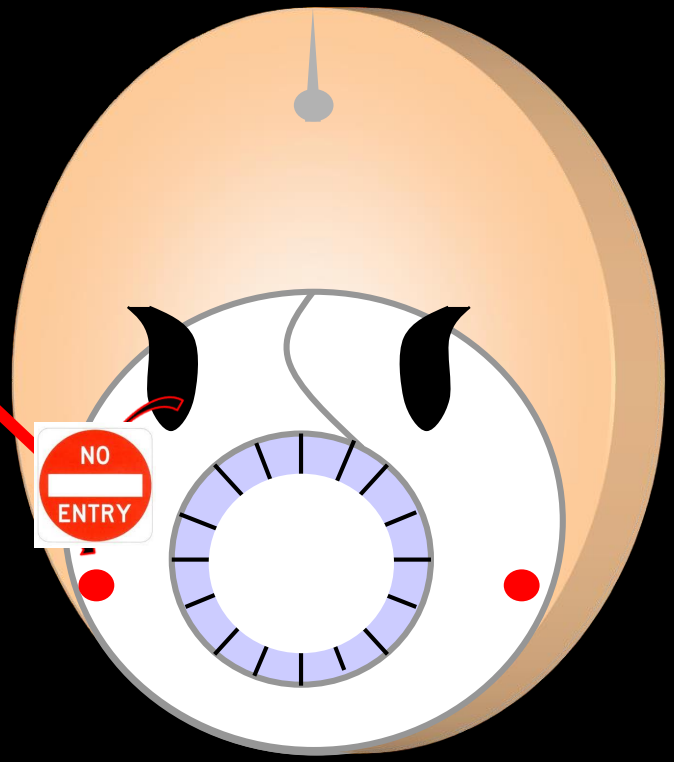


Wong et al, 2013

A new approach to sterility 2: disrupt the early migration of the PGCs

Migrating primordial germ cells

Sdf-1a gradient



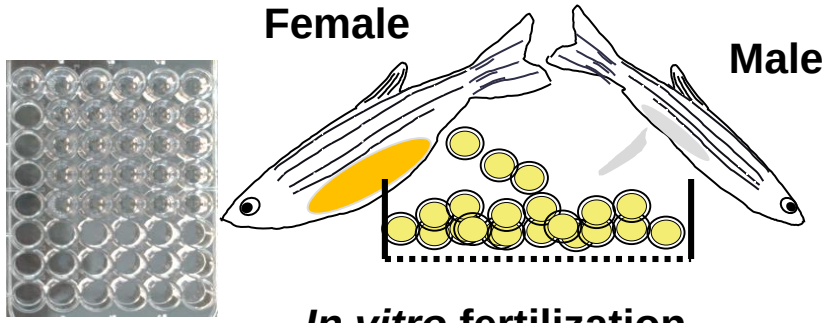
No PGC migration

No gonads

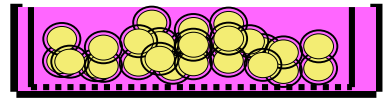
The Strategy For Developing Non-GM Sterile Fish

- **Use Tg fluorescent zebrafish (GnRH, PGCs) to screen (via immersion) for silencing compounds that disrupt the development of the GnRH system or PGCs and induce sterility**
- **Determine minimal doses, duration and timing of immersion**
- **Select compounds and conditions to apply in trout/salmon**

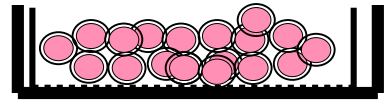
Screening for compounds that disrupt GnRH3 or PGC development in the G/R-FP zebrafish model



In vitro fertilization



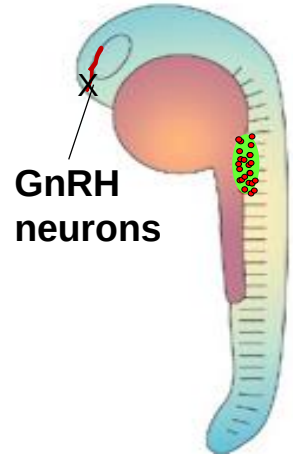
Immersed in compound for 5 - 24 hours



Wash/egg water

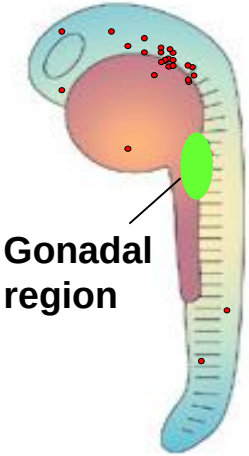
X GnRH development

X PGC development



GnRH neurons

and or



Gonadal region

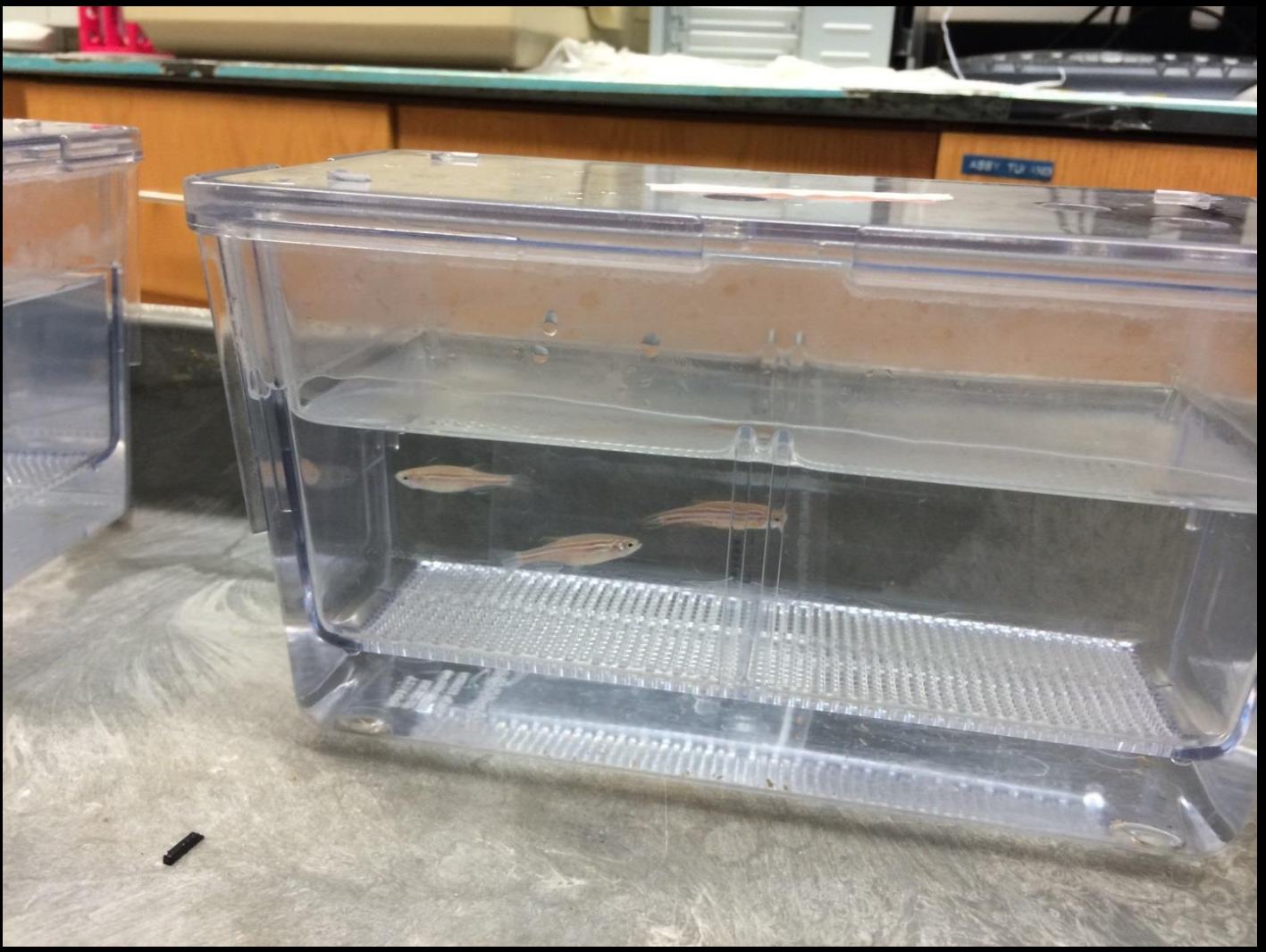
Adult infertile fish

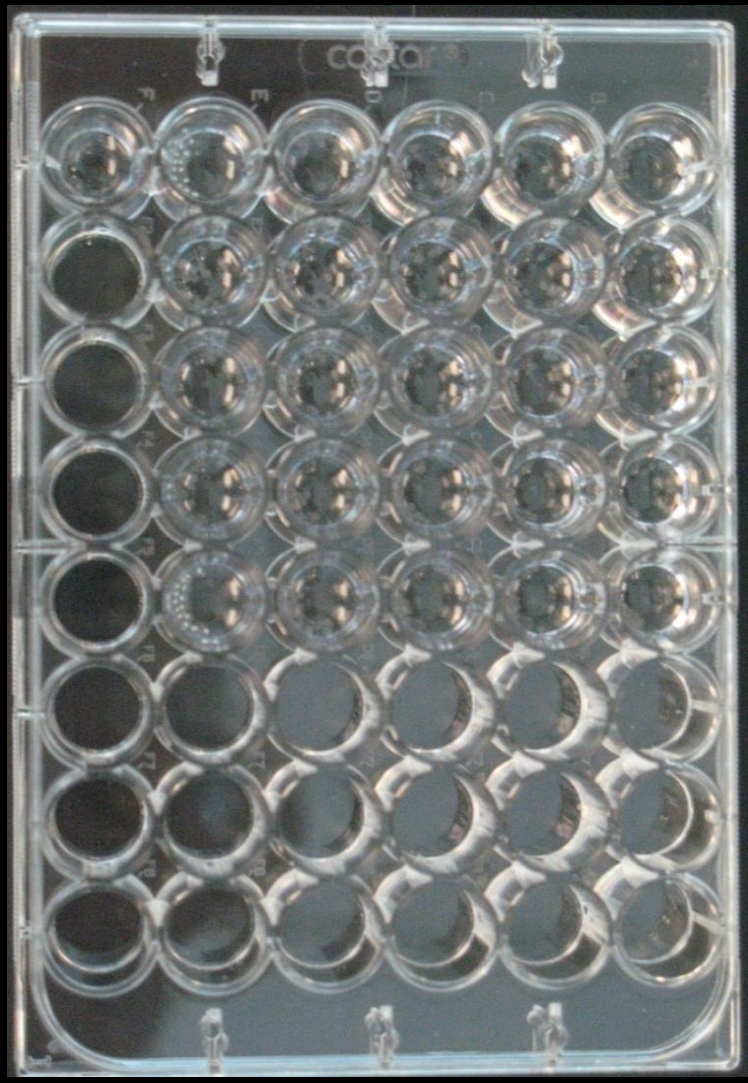
No fertile gonadal development

Determine minimal doses, duration and timing of immersion

Arianna Servili; Ten-Tsao Wong

Screening for compounds that disrupt GnRH3 or PGC development in the G/R-FP zebrafish model





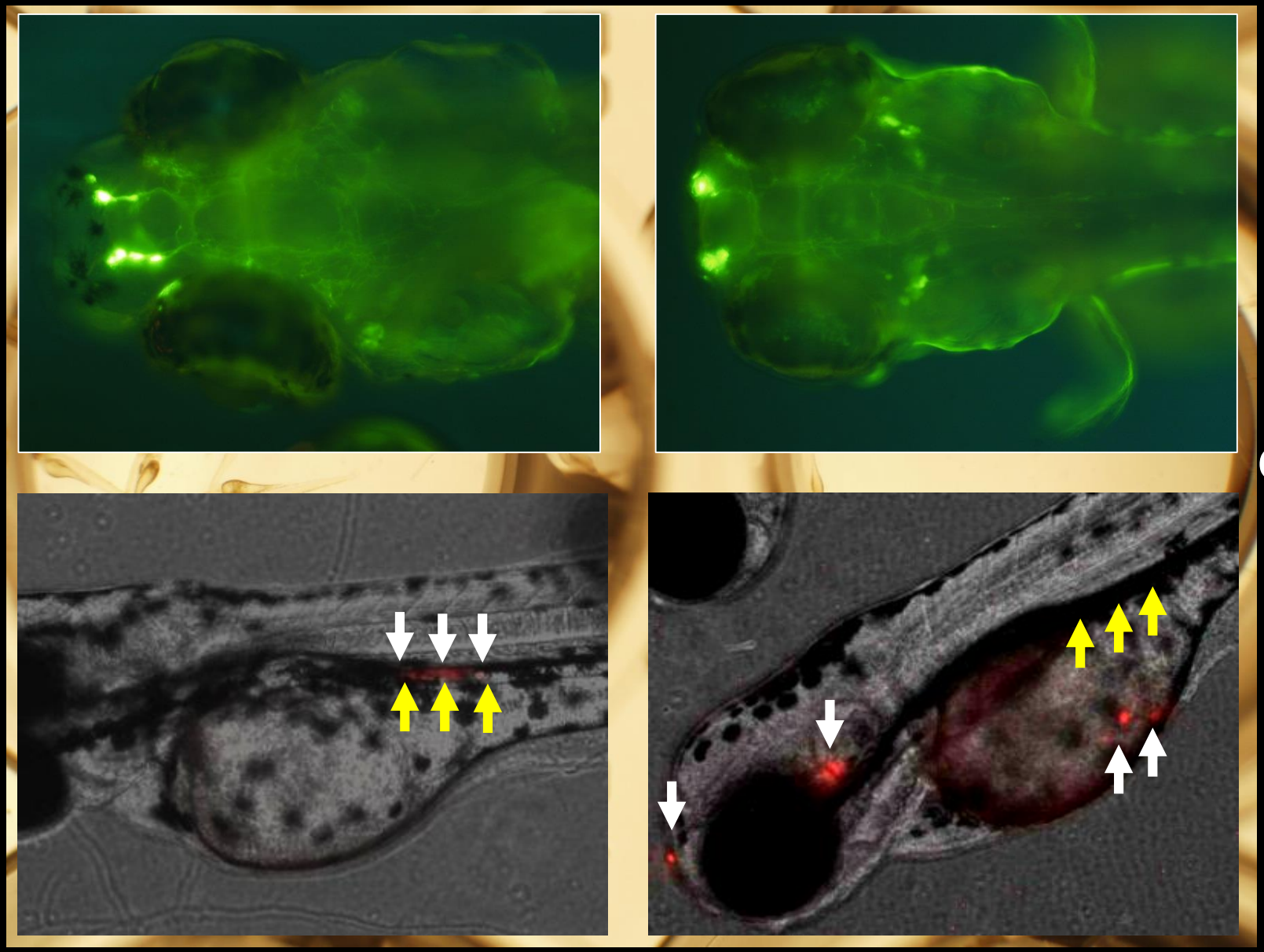
Screening for compounds in the zebrafish model

GnRH3

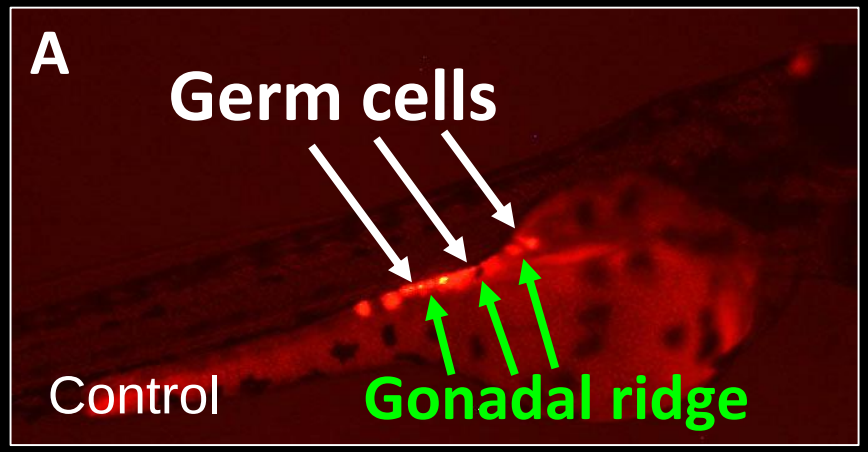
Control

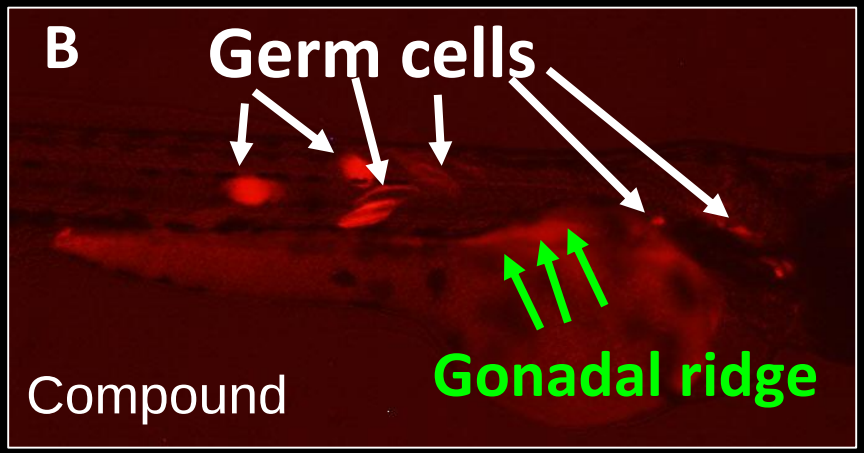
PGCs

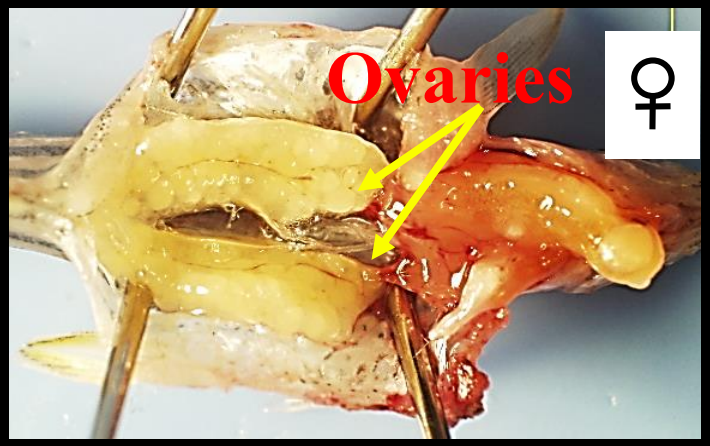
Compound

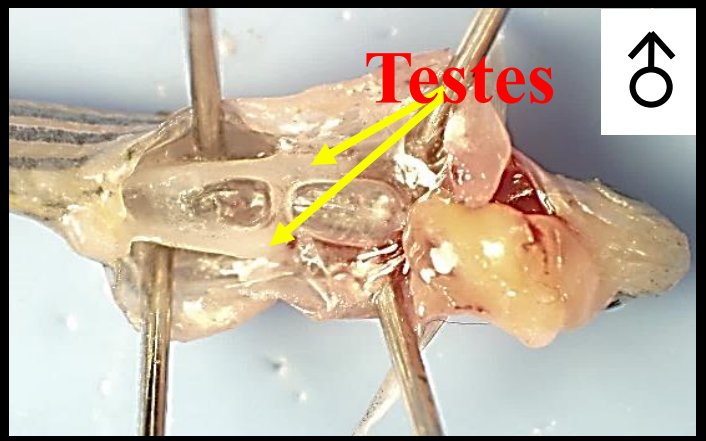


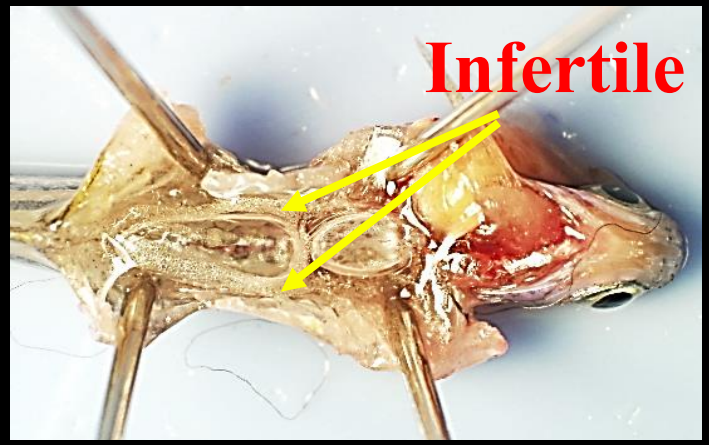
Immersion in PGC silencing compounds led to their mis-migration and 100% sterility in zebrafish







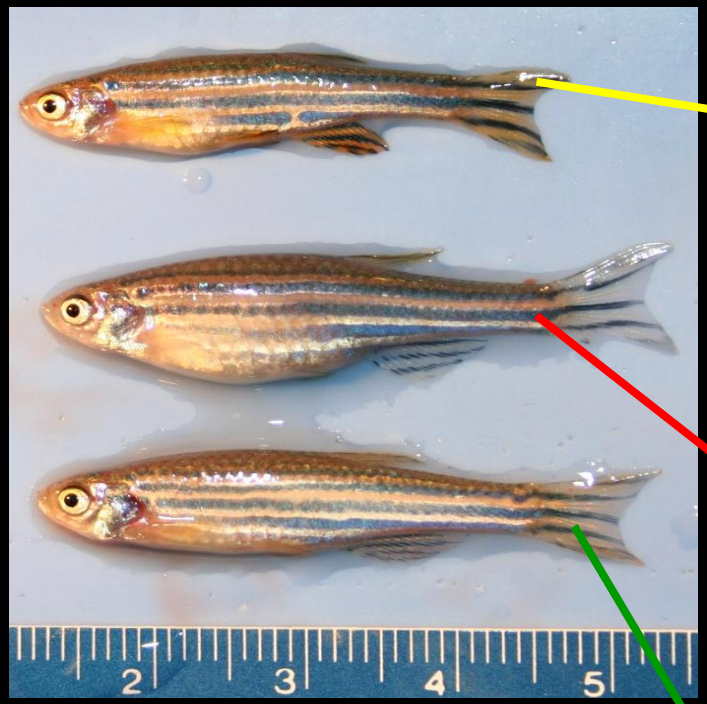


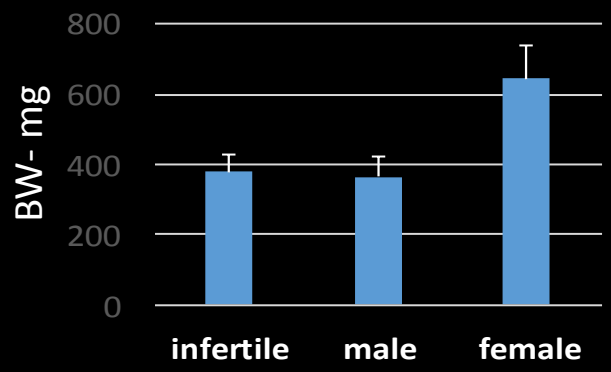


Sdf1; Cxcr4; Dnd; Nanos.....

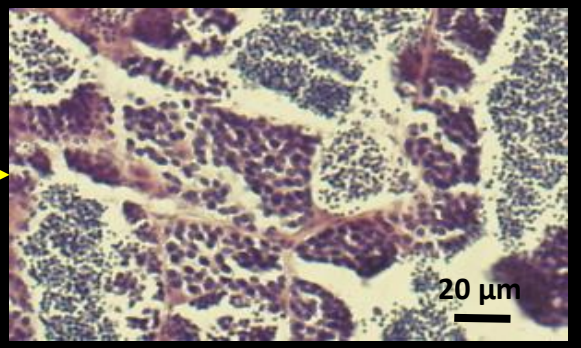
Wong and Zohar, 2014, 2015

Immersing zebrafish embryos in Dnd-MO-Vivo for 5 hours induced 100% sterility

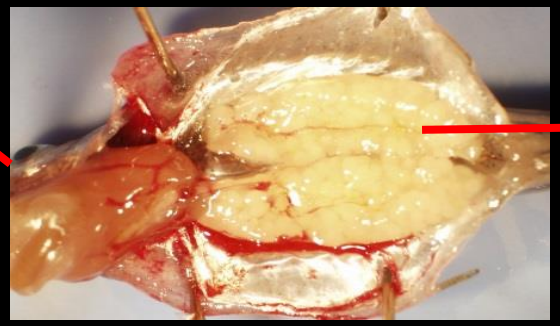


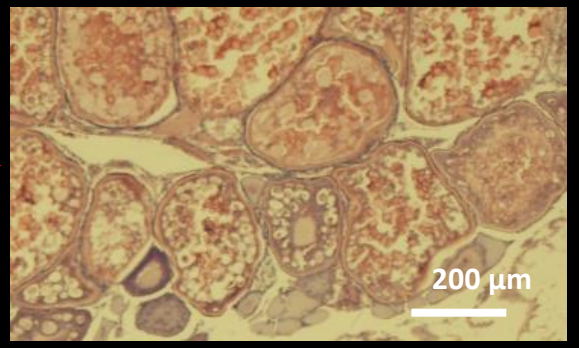


Control male

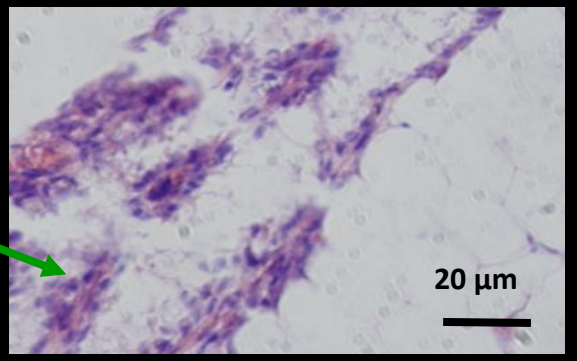


Control female





Infertile fish



Wong and Zohar, 2014, 2015

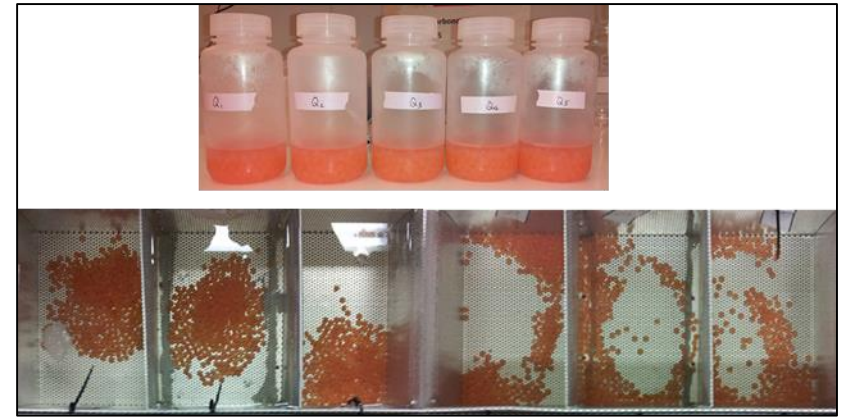
Implementing the findings in trout and Atlantic salmon

USDA-WV

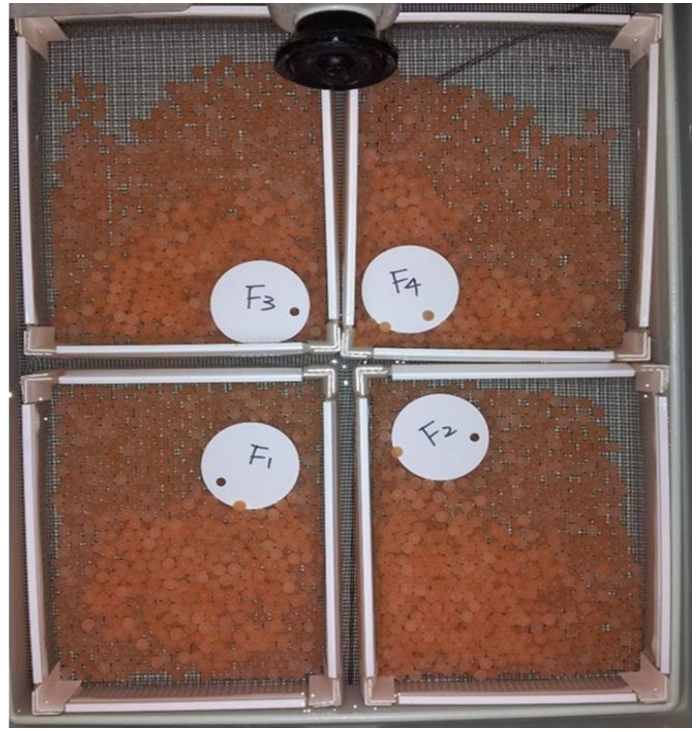
Troutlodge

USDA-ME

A recent scale-up trial at AquaGen



Atlantic salmon- monitoring performance to maturity: survival, growth rate, stress tolerance- fresh water and cages



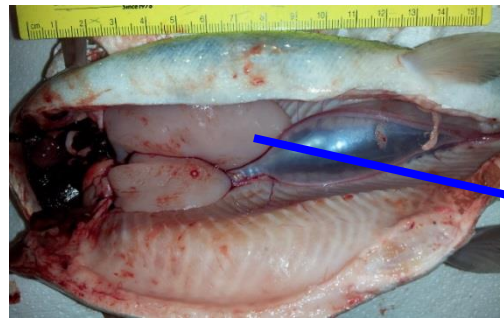
Production of sterile rainbow trout

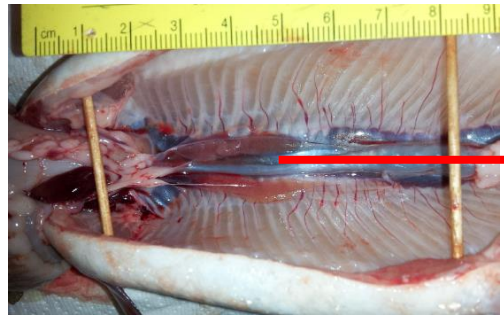
14 months old (48 hour immersion in Dnd-MO-Vivo)

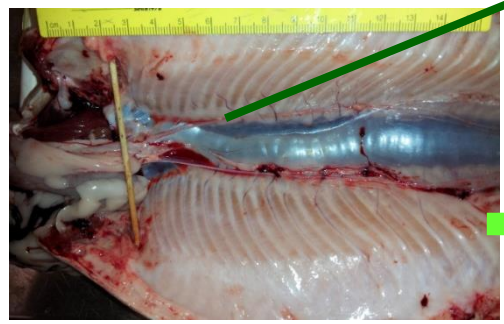
Male Control

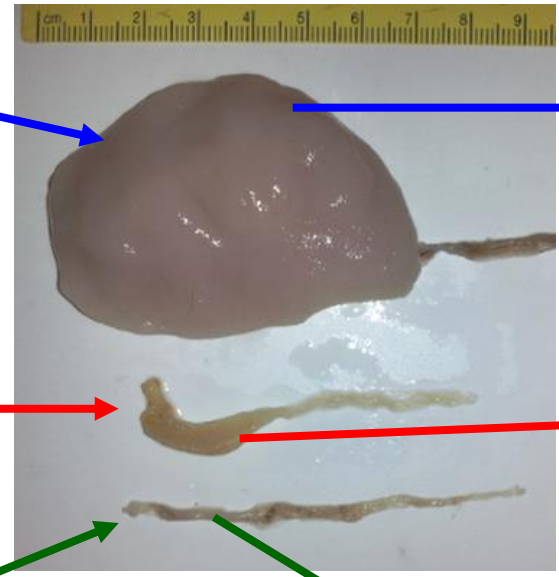
Female Control

Infertile fish Treated

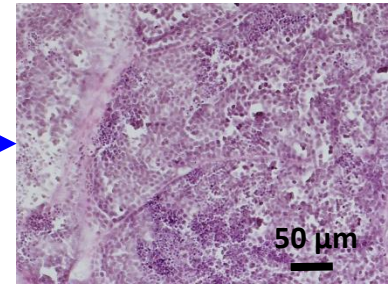


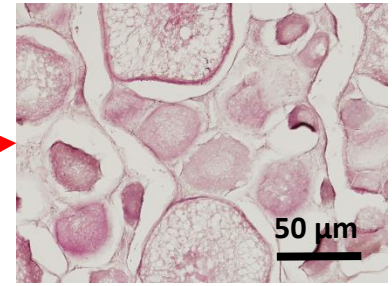


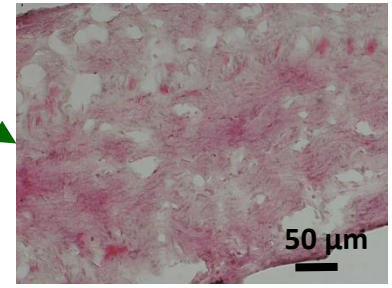




Infertile fish

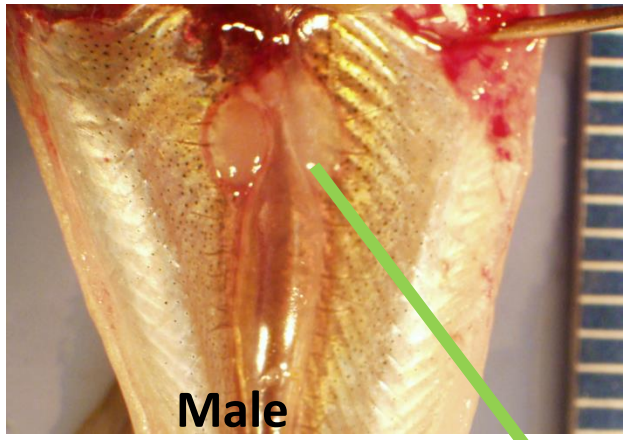


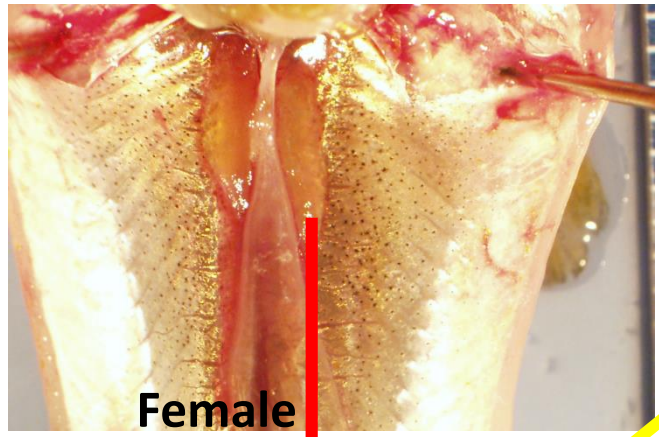


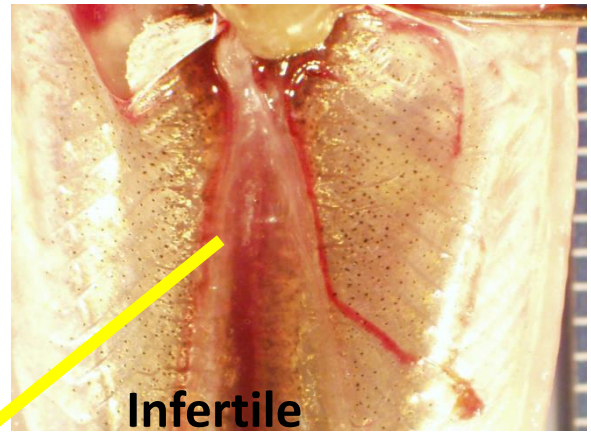


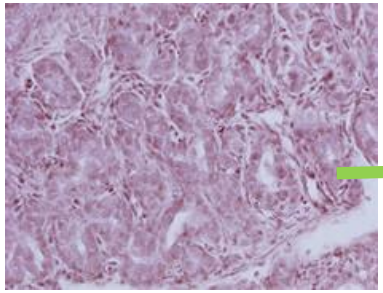
Production of sterile Atlantic salmon

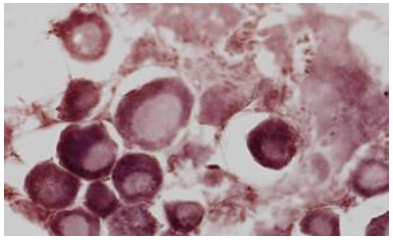
9-10 months old

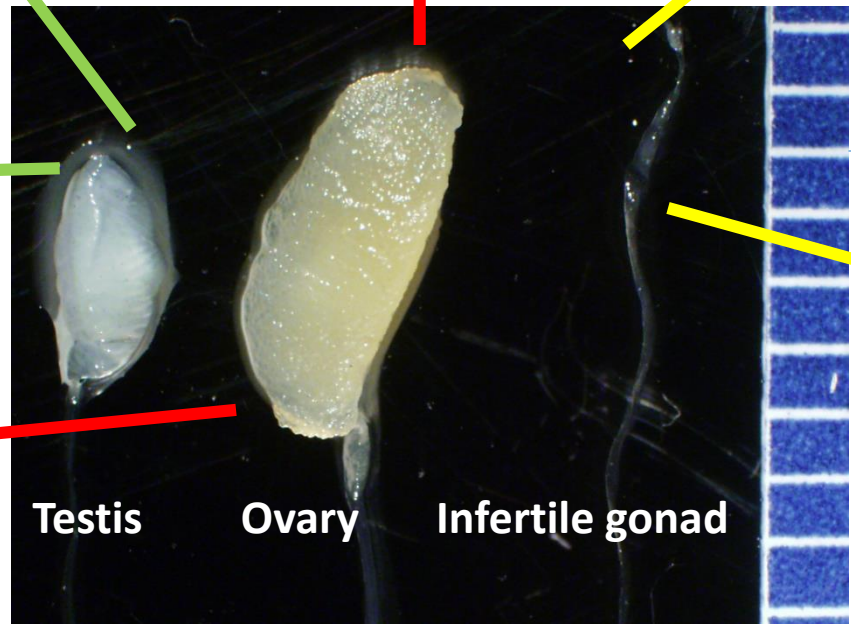




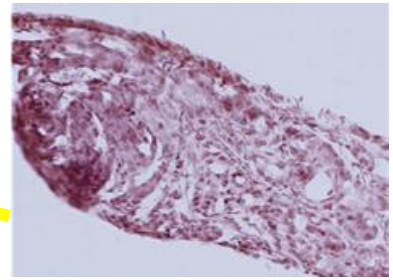






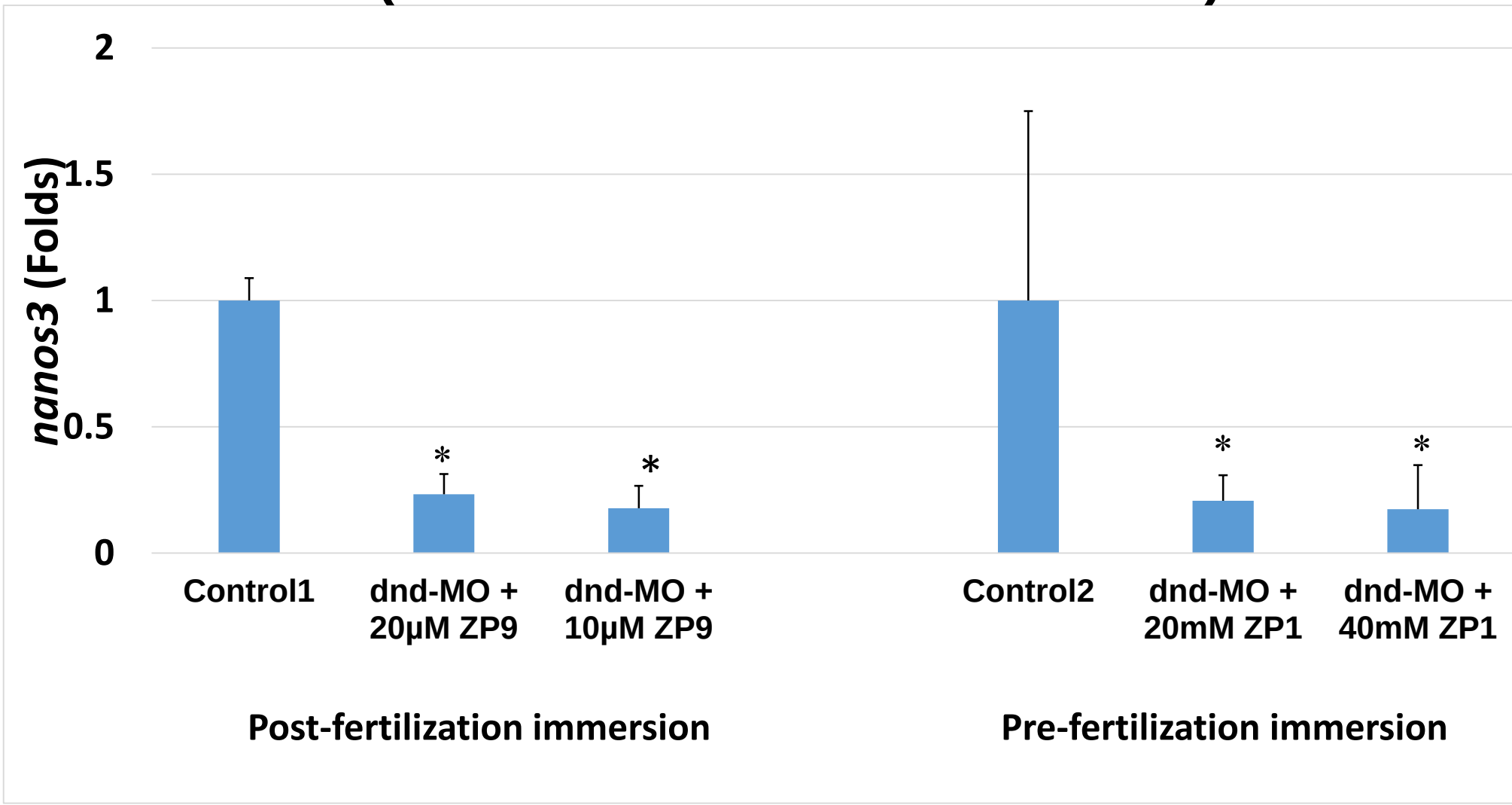


1 mm

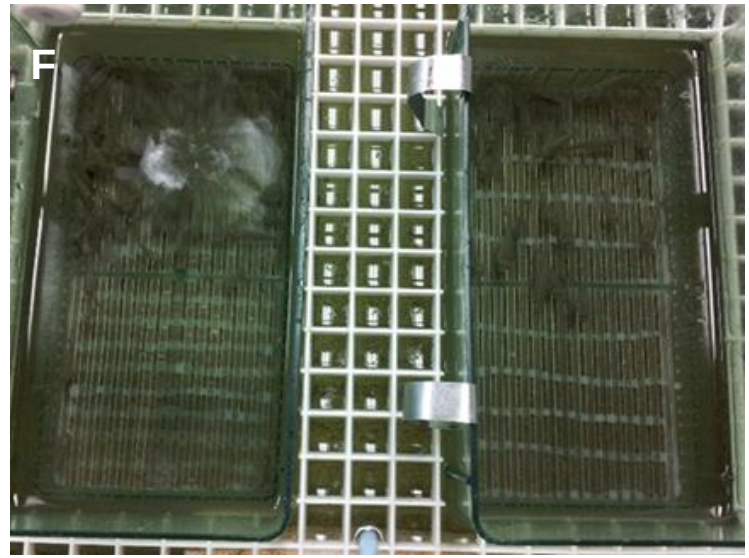


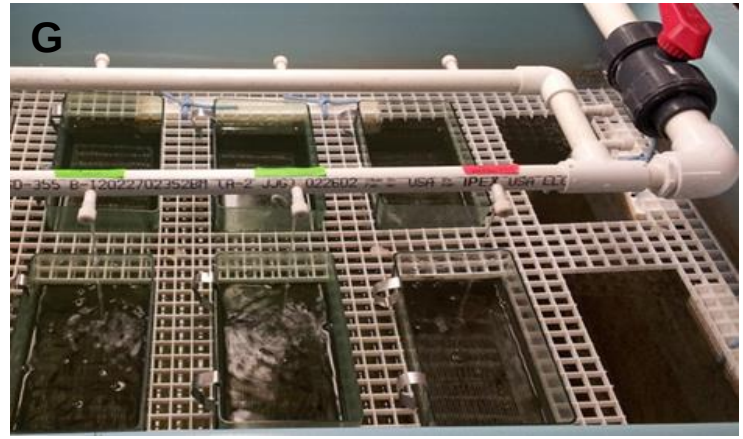
The search for early indicators of sterility: nanos3- a germ cell marker

(Atlantic salmon- 2.5 weeks PH)



Tilapia *dnd*-MO (t*dnd*-MO) disrupted PGC development in tilapia larvae





In Conclusion:

- Silencing of genes responsible for early migration of GnRH3 neurons and PGCs disrupt the development of both systems
- The GnRH3 system is very robust, plastic and recovers from early arrest of migration resulting fertile fish
- Mismigrating PGCs do not make it to the gonad leading to reproductively sterile fish
- 5 hour immersion in Dnd-Mo-Vivo leads to 100% sterility in zebrafish with no effect on performance; 48 hour immersion is effective in rainbow trout and Atlantic salmon; Promising early results in tilapia
- Germ cell markers are making it possible to quickly optimize sterility protocols in salmonids and other farmed species

Acknowledgements

DMB/IMET/UMBC

Dr. Arianna Servili

Dr. Nilli Zmora

Mr. John Stubblefield

Mr. Steve Rogers , ARC team

Troutlodge

Mr. Jim Parsons

Mr. Kyle Martin

Mr. Douglas Dixon

AquaGen

Dr. Nina Santi

Dr. Maren Mommens

Ms. Mona Rostad

National Cold Water Marine Aquaculture Research Center, USDA-ARS, ME

Dr. Bill Wolters

Dr. Brian Peterson

Dr. Garry Burr

Ms. Melissa Milligan

Cool and Cold Water Aquaculture Research Center, USDA-ARS, WV

Dr. Greg Weber

Mr. Josh Kretzer

ACD Pharmaceuticals

Ms. Mariann Dønnum

Europharma

ACDPHARMA
INNOVATIONS IN AQUACULTURE

MABIT
Et næringsrettet FoU-program innen marin bioteknologi i Nord-Norge

USDA
Biotechnology Risk Assessment Program

11th IMBC 2016

International Marine Biotechnology Conference

"Health, Wealth, and Innovation"

August 29 – September 2, 2016

Baltimore, MD, USA

More information can be found at www.theimba.org