## Production of Reproductively Sterile Fish to Eliminate Maturation

#### **Ten-Tsao Wong and Yonathan Zohar** Institute of Marine and Environmental Technology University of Maryland, MD, USA



## Sterility in Farmed Fish: the Rationale

- Achieve better somatic growth (no early maturation)
- Prevent deterioration of flesh quality and mortality
- Protect IP strains
- Biological containment: prevent propagation of farmed/domesticated, non-native and GM fish



## Atlantic salmon escape event in the Pacific

FOOD FOR THOUGHT

#### 'Environmental Nightmare' After Thousands Of Atlantic Salmon Escape Fish Farm

August 24, 2017 - 10:52 AM ET



FROM EAR



#### Farmed fish should be sterile

Business | Environment | Local News | Puget Sound

#### After Atlantic salmon spill, fish farms' future under attack on both sides of border

Originally published September 1, 2017 at 6:00 am | Updated September 1, 2017 at 8:59 pm





#### 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







48 hrs







## Early Migration of Primordial Germ Cells (PGCs) Dnd protein Migrating primordial germ cells

PGCs are reproductive "stem" cells that migrate to the developing gonads; the Deadend (Dnd) protein is essential for that migration

Gonad

Modified from G. Yoshizaki

## Disrupt the early migration of the PGCs





#### Transgenic zebrafish with RFP in Primordial Germ Cells for visualizing disruption



#### The Strategy For Developing Non-GM Sterile Fish

- Use red fluorescent zebrafish (PGCs) to screen (via immersion) for silencing compounds (MO) that disrupt the migration of the 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 PGC development in the RedFP zebrafish model



# Screening for compounds that disrupt PGC development in the R-FP zebrafish model



#### Screening for compounds in the zebrafish model





# Immersion in Dnd-MO-Vivo for 5 hours led to PGC mis-migration and 100% sterility in zebrafish





#### Wong and Zohar, 2014, 2015

#### Implementing the findings in trout and Atlantic salmon

#### USDA- WV Troutlodge- WA USDA- ME AquaGen- Norway



#### **Production of sterile rainbow trout**

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



**Infertile fish** 

# Production of sterile rainbow trout using pre-fertilization immersion



#### **Production of sterile Atlantic salmon**

9-10 months old



#### Current trials: Optimization of Conditions For 100% sterility

Atefetitizizationimmeerisionfo(gelealedebgss) for 24-48 hrs

- Micropyle open
- -Petrateable takorion
- -Befige uptakening
- Lower doses



Dnd anti-sense (MO) Molecular transporter Vivo/ZP9

#### In Conclusion:

- Mismigrating PGCs do not make it to the gonad, resulting in 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 and sablefish
- Germ cell markers make it possible to quickly optimize sterility protocols
- Accelerating studies, large scale optimization and performance trials (Riverence; EvAqua)

#### **Many Collaborations**

National Center for Cool and Cold Water Aquaculture Research (ARS/USDA) Greg Weber, Josh Kretzer, Beth Cleavland

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Riverence

Rob Young, Bob Iwamoto





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



**Biotechnology Risk Assessment Program** 

# Thank you

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