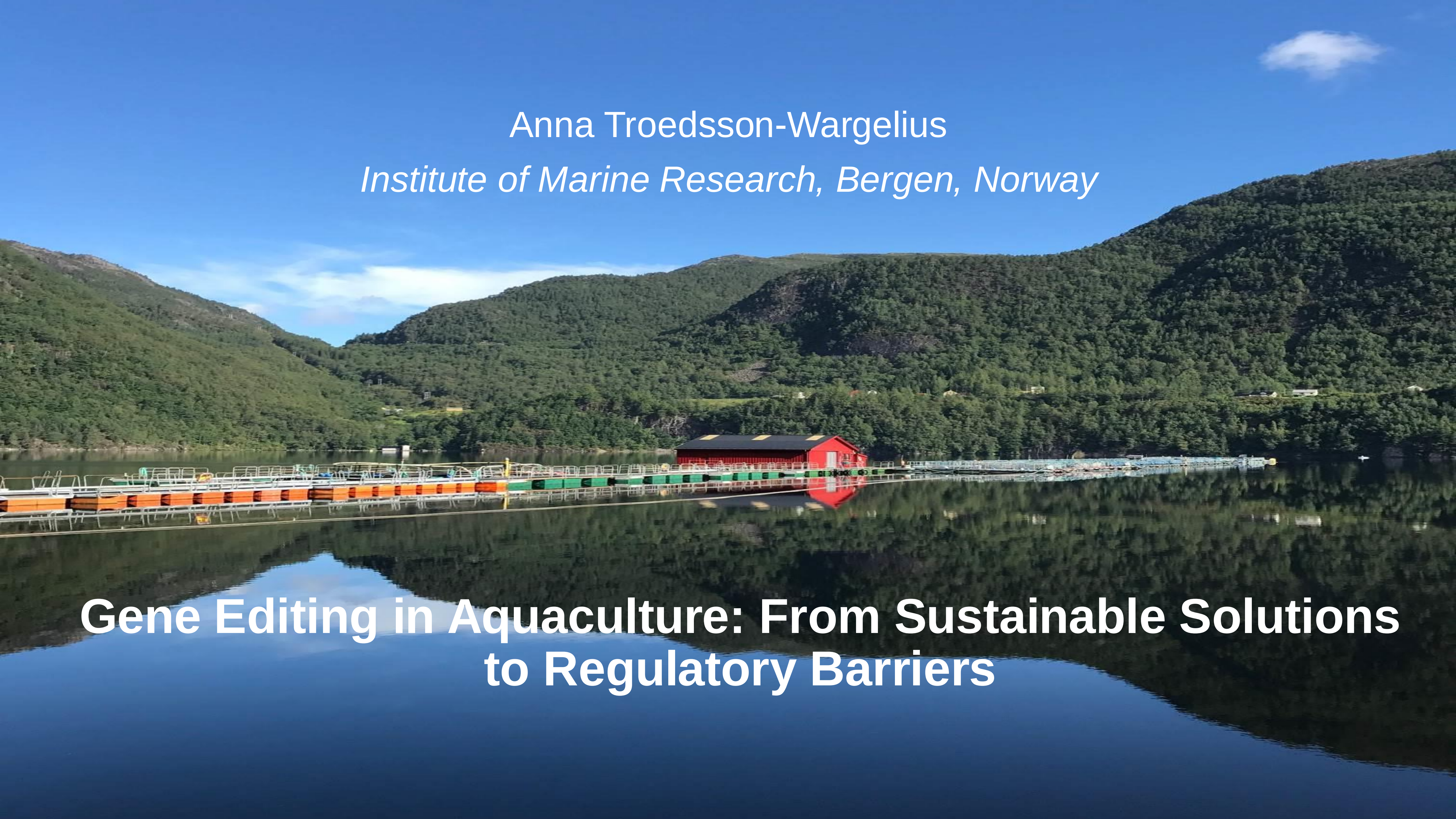
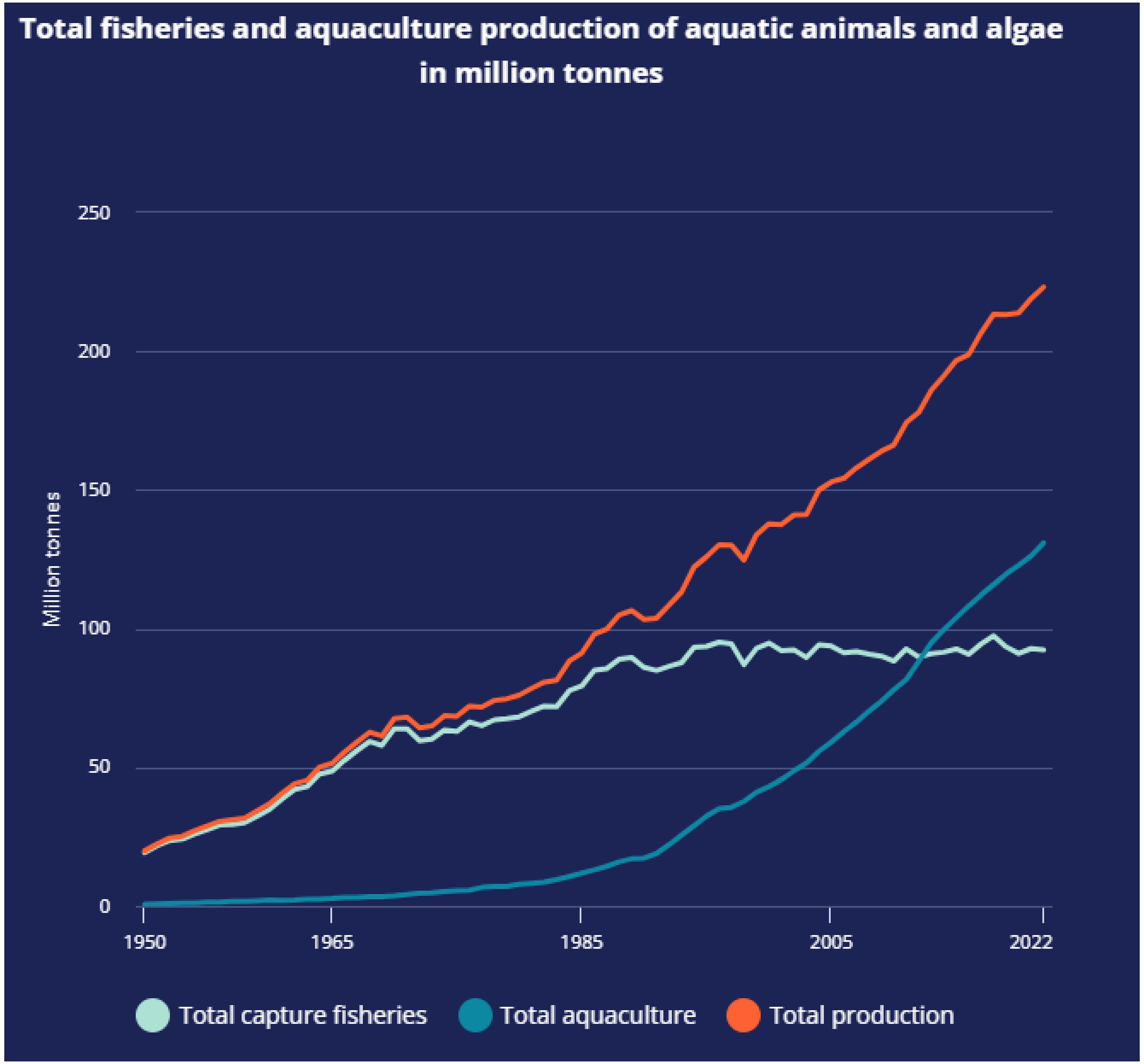


Anna Troedsson-Wargelius

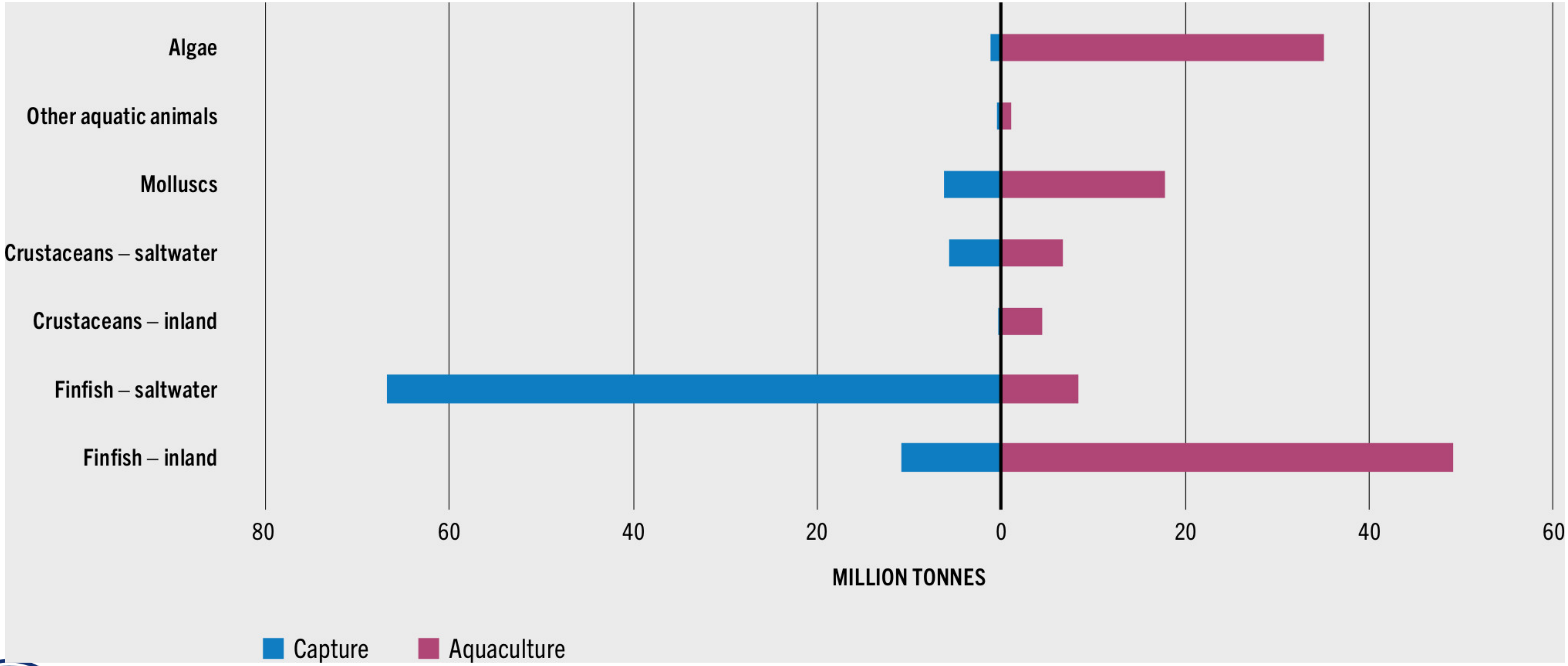
Institute of Marine Research, Bergen, Norway

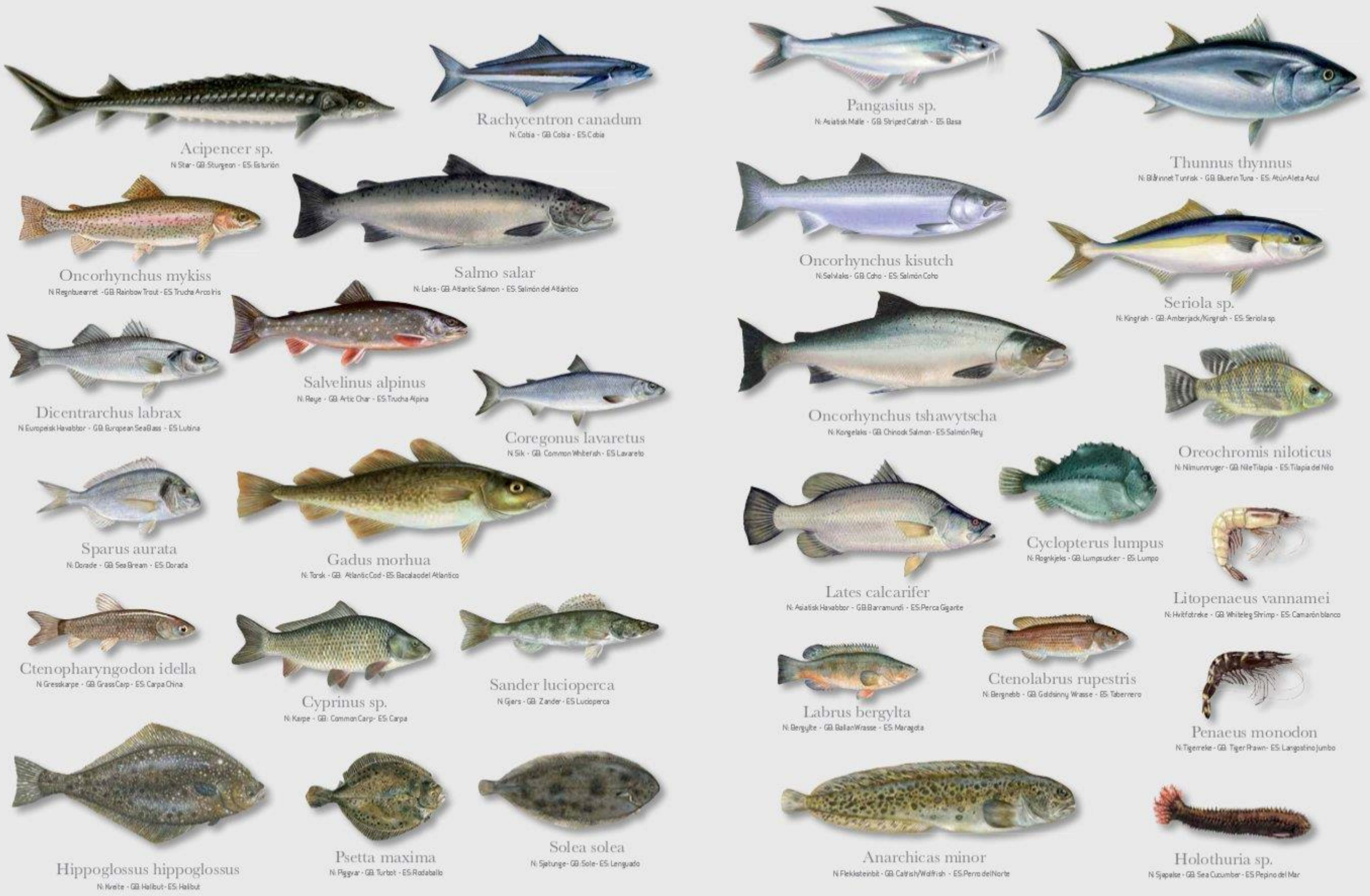
Gene Editing in Aquaculture: From Sustainable Solutions to Regulatory Barriers



Food and Agriculture Organization of the United Nations

THE STATE OF
WORLD FISHERIES
AND AQUACULTURE
2024
BLUE TRANSFORMATION IN ACTION

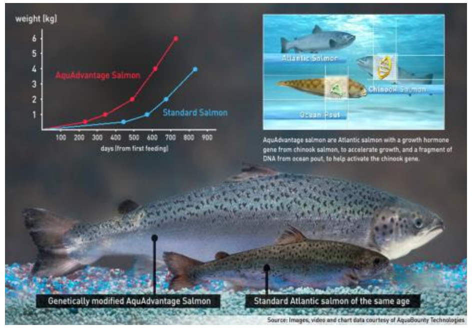

Algae
Other aquatic animals
Molluscs
Crustaceans – saltwater
Crustaceans – inland
Finfish – saltwater
Finfish – inland
80
60
40
20
0
20
40
60
MILLION TONNES
Capture
Aquaculture



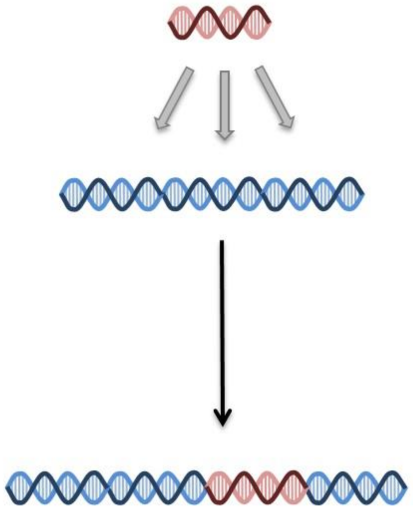
INSTITUTE OF MARINE RESEARCH

Gene editing: CRISPR/Cas9

Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR associated protein 9

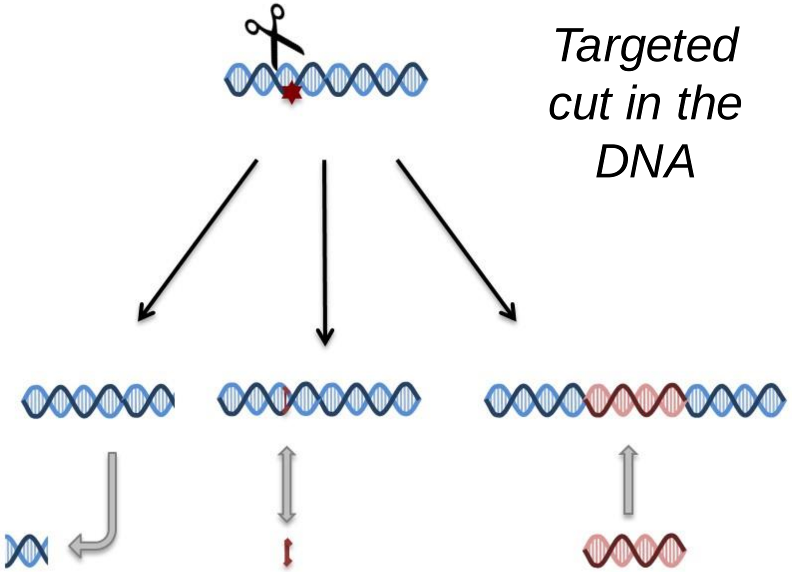


Traditional genetic engineering

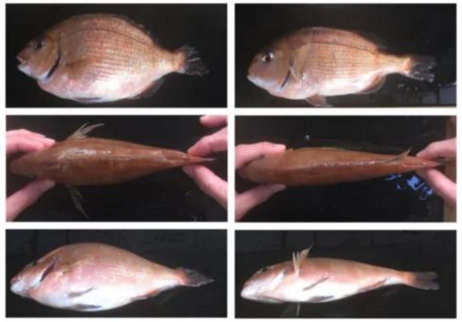


Insert new DNA, random location

Genome editing (using CRISPR)



Targeted cut in the DNA



Remove DNA

Exchange DNA

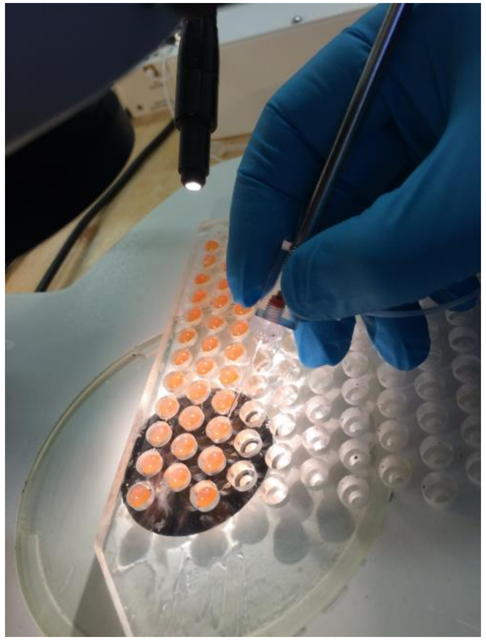
Add DNA, specific location

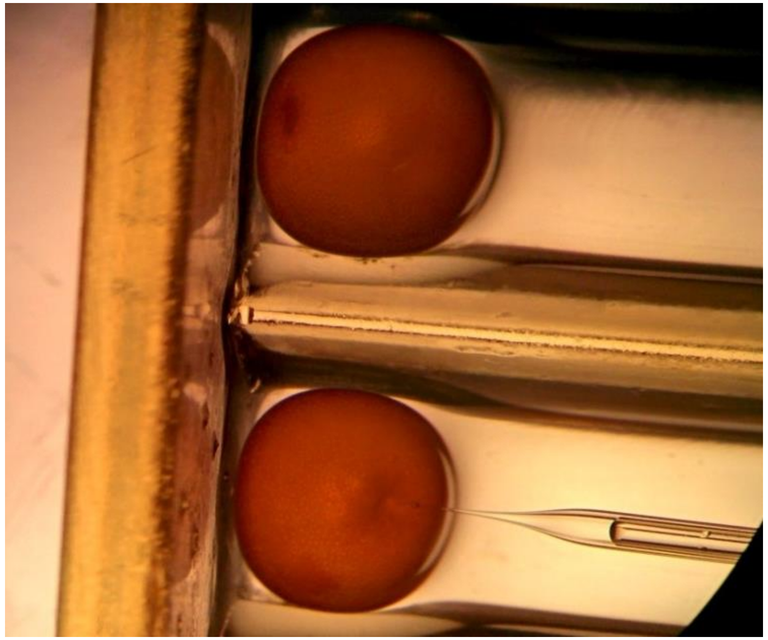
Modified from: https://www.bioteknologiradet.n

How does gene editing in farmed fish differ significantly from other farmed animals?

- Oviparous
- High fecundity
- Often have duplicated genes
- Water living
- Long generation time

Generic methods for introduction of edits in fish





Injection of guide RNA + Cas9 in newly fertilized eggs
Targeting a pigmentation gene: *slc45a2* (*albino*)

Successful editing = F0 albinos

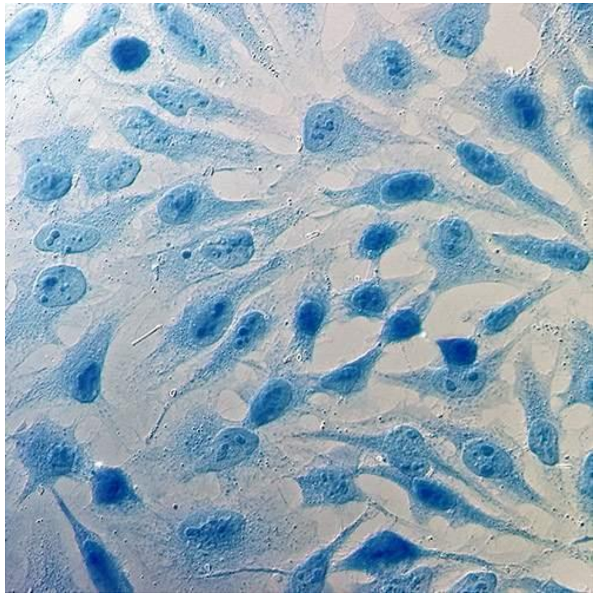
Alternatives
-Sperm electroporation
-Cell lines/surrogacy

Cell lines

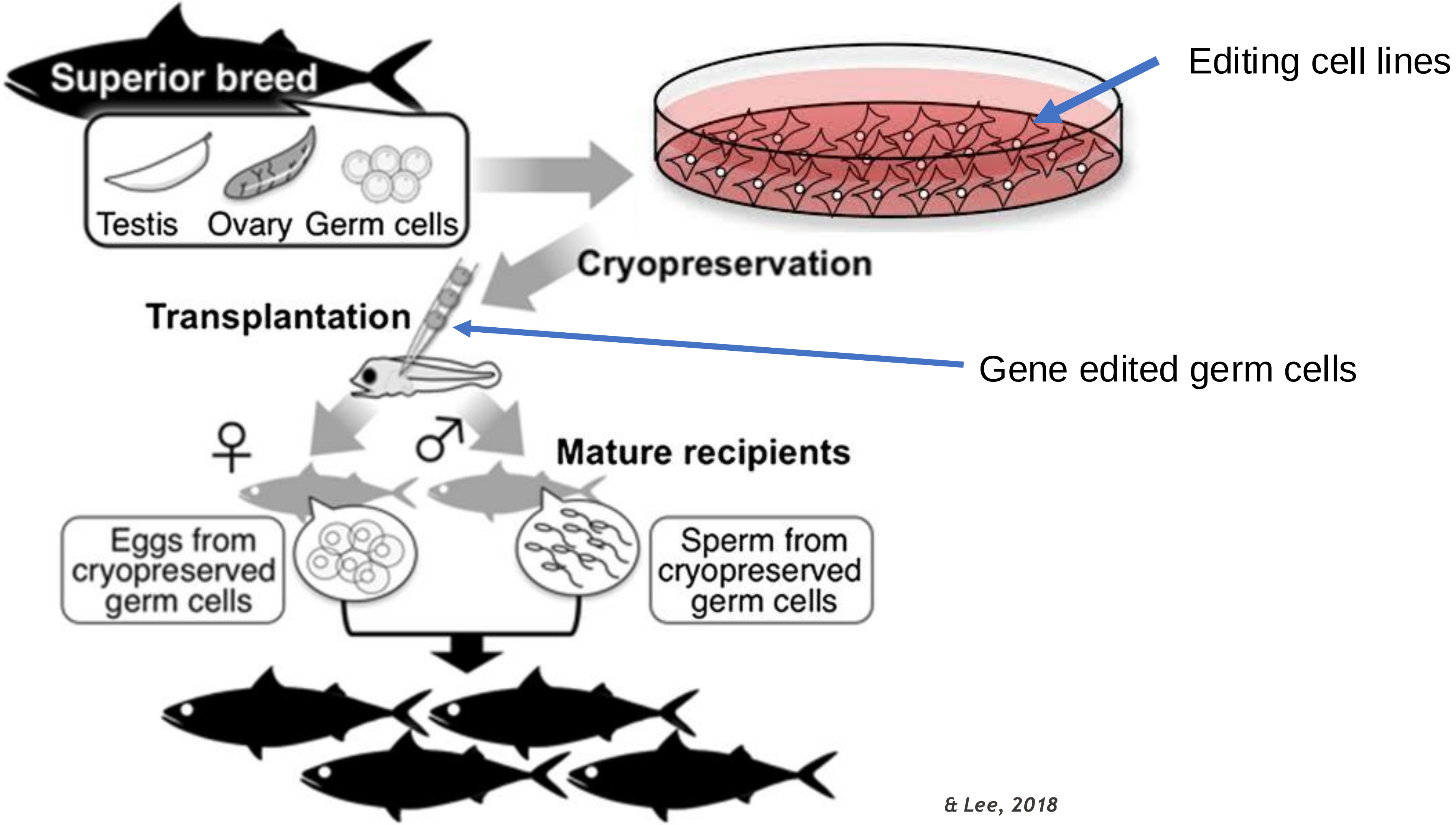
-used for research, disease susceptibility

-can be used to develop cell lines, that are useful for vaccine production

- germ cells may also be cultured

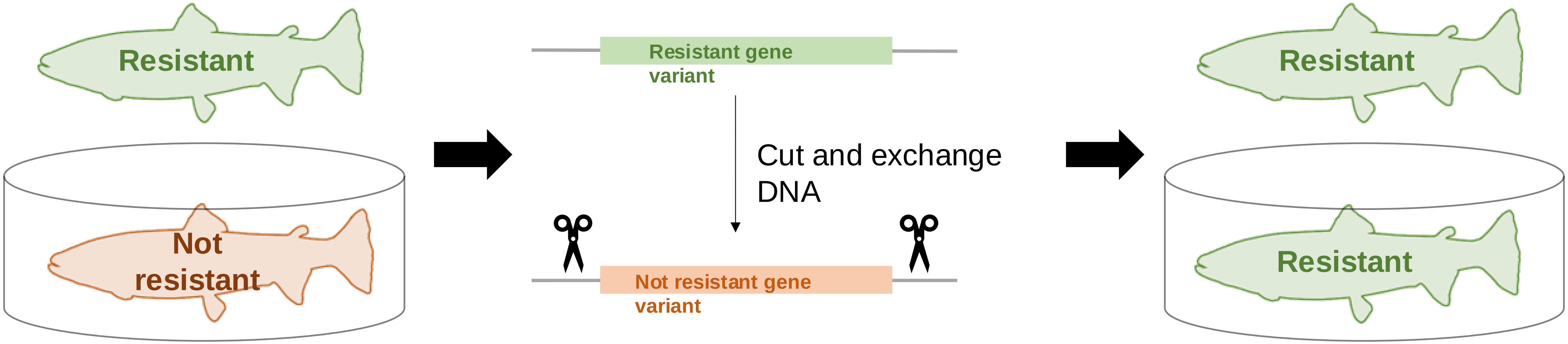


Surrogate broodstock technology



& Lee, 2018

Edit for disease resistance



Areas of interest to use the technology in aquaculture

Production traits- growth, filet quality, color etc

Reproduction- sterility, maturation and breeding

Disease resistance - parasites virus and bacteria

New cell lines- to be able to produce better vaccines

Sustainable feeds-Locally grown, lower trophic levels

Traits	Function	Genes	Species
Growth	Double muscle	*mstn*	Yellowhead catfish, *Eurasian carp*, **Red sea bream**, *Olive flounder*, *Nile tilapia*, *Channel catfish*, *Large yellow croaker*, *Blotched snakehead*
	Appetite	*lepr*	***Tiger pufferfish***
	Appetite	*mc4r*	*Channel catfish*
	Metabolism	*pi3k*	*Gibel carp*
	Growth	*igfbp*	*Rainbow trout*
Reproduction	Sterility	*lh*	*Channel catfish*
	Sterility	*fshr*	*Atlantic salmon*
	Sterility	*bmp15*	*Atlantic salmon*
	Sterility	*eef1a*	*Nile tilapia*
	Sterility, Surrogacy	*piwil2*	*Nile tilapia, Atlantic salmon*
	Sterility, Surrogacy	*dnd*	***Atlantic salmon***, *Rainbow trout*
	Sex det.	*cyp19a1a* and *foxl2*	*Swamp eel*
	Sex det.	*cyp17a1*	*Eurasian carp*
	Sex det.	*amhy*	*Nile tilapia*
	Sex det.	*pfpdz1*	*Yellow catfish*
Disease resistance	Virus suscep./innate immunity	*viperin*	*Gibel carp*
	Ammonia resistance/innate immunity	*chop*	Pond loach
Meat quality	**Omega-3**	*fads2*	*Atlantic salmon*
	Omega-3	*elovl2*	*Atlantic salmon*, *Channel catfish*
	Omega-3	*fat-1* and *fat-2*	*Channel catfish*

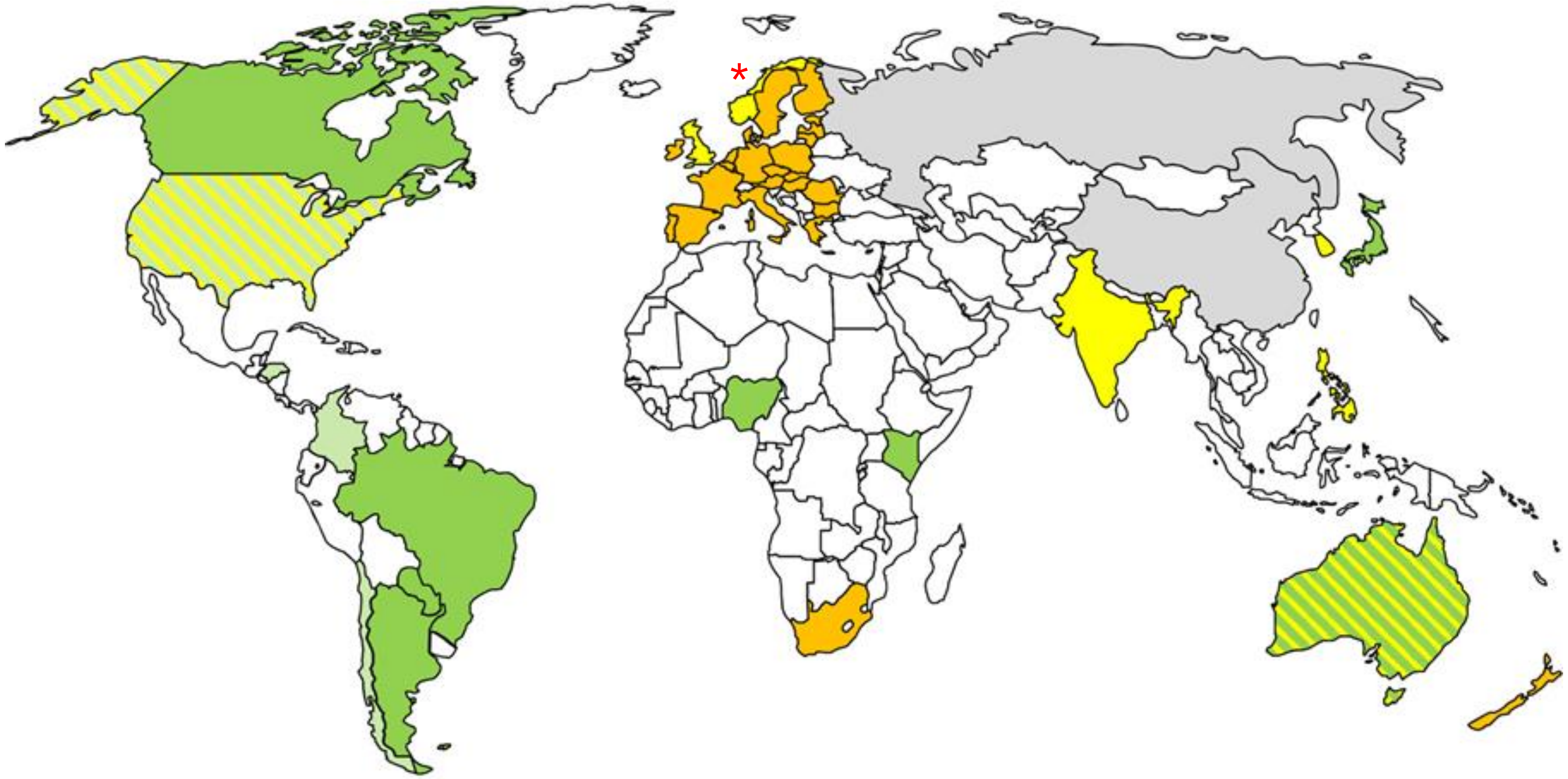
News in Brief | Published: 30 December 2021

Japan embraces CRISPR-edited fish

Nature Biotechnology **40**, 10 (2022) | Cite this article

How could we move thes fish into sea cages

Filet quality



Countries with regulatory policy with exclusions

Countries with regulatory policy with exclusions (plants only)

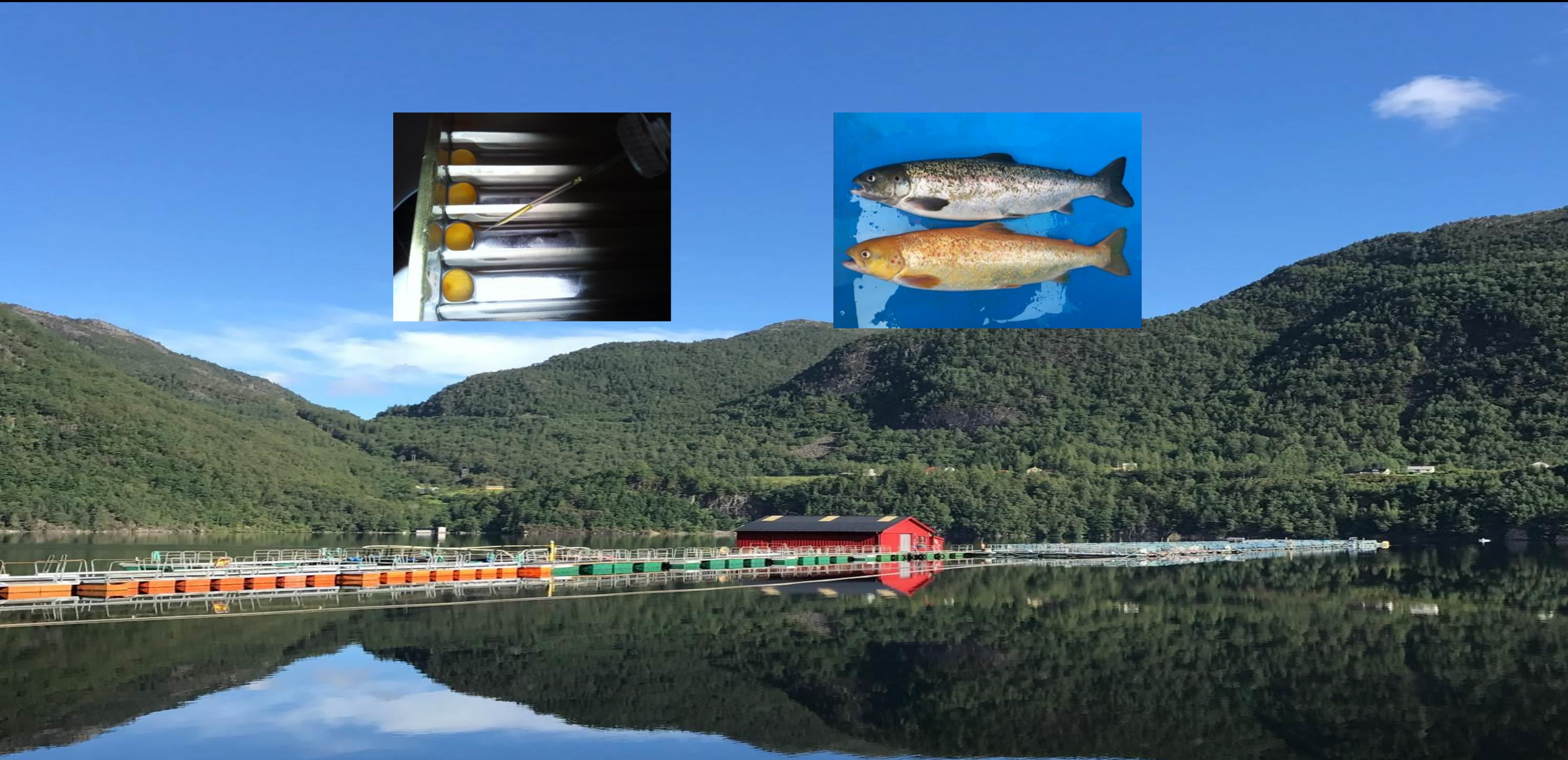
Countries with pending policies, regulations, or legal rulings

Countries with GMO only policy with no exclusions

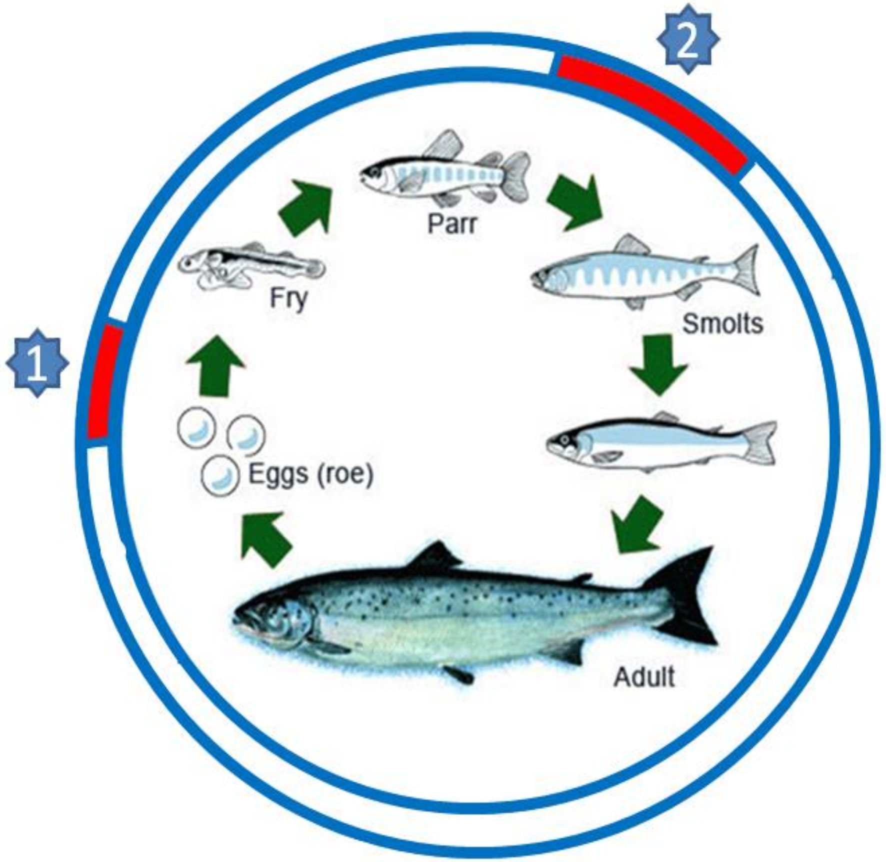
Towards production of genome-edited aquaculture species

Eric M. Hallerman, Rex Dunham, Ross D. Houston, Mark Walton, Anna Wargelius, Diane Wray-Cahen

Moving sterile Crispr salmon experiments from tank to sea cages trial-regulatory barriers



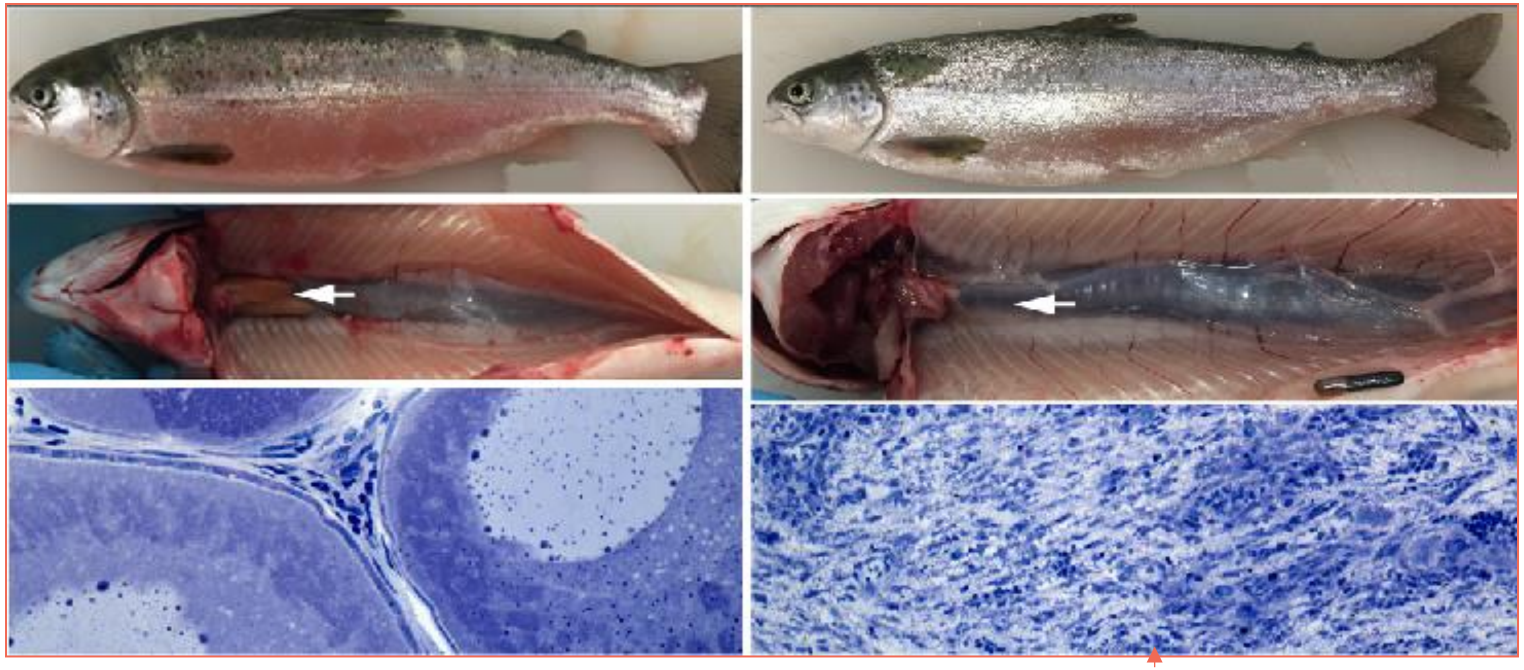
Sterile salmon by gene editing



1) Inhibit germ cell formation
2) Prevent maturation

Sterile Salmon

by Crispr-cas9 for knocking out *dead end* (*dnd*) gene



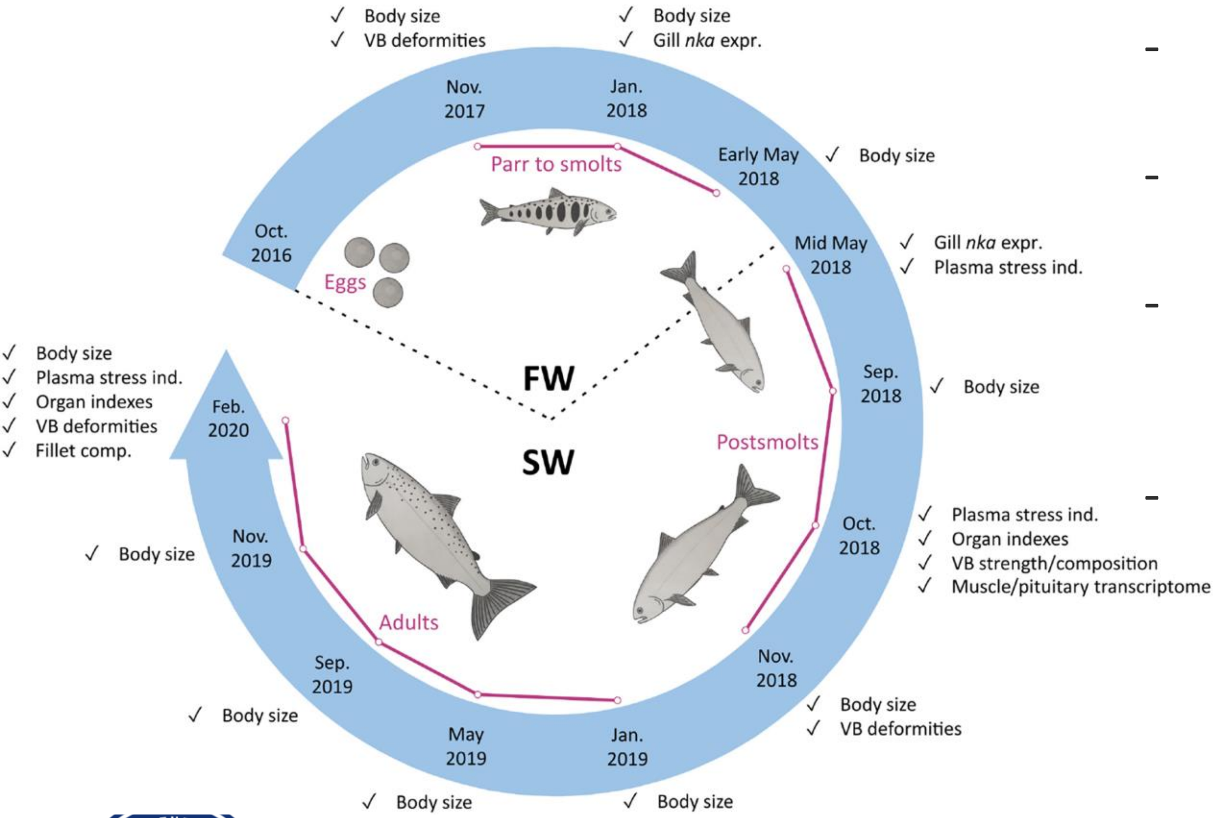
Wargelius et al *Sci. rep.* 2016

Kleppe et al. *Sci. rep.* 2017

NO PUBERTY

100% GERM CELL FREE

dndKO vs WT salmon – conclusions

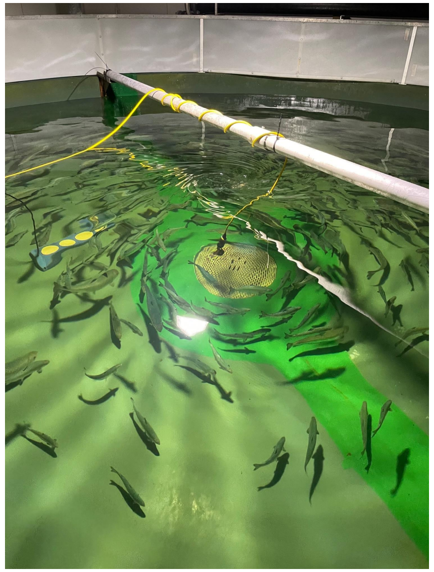


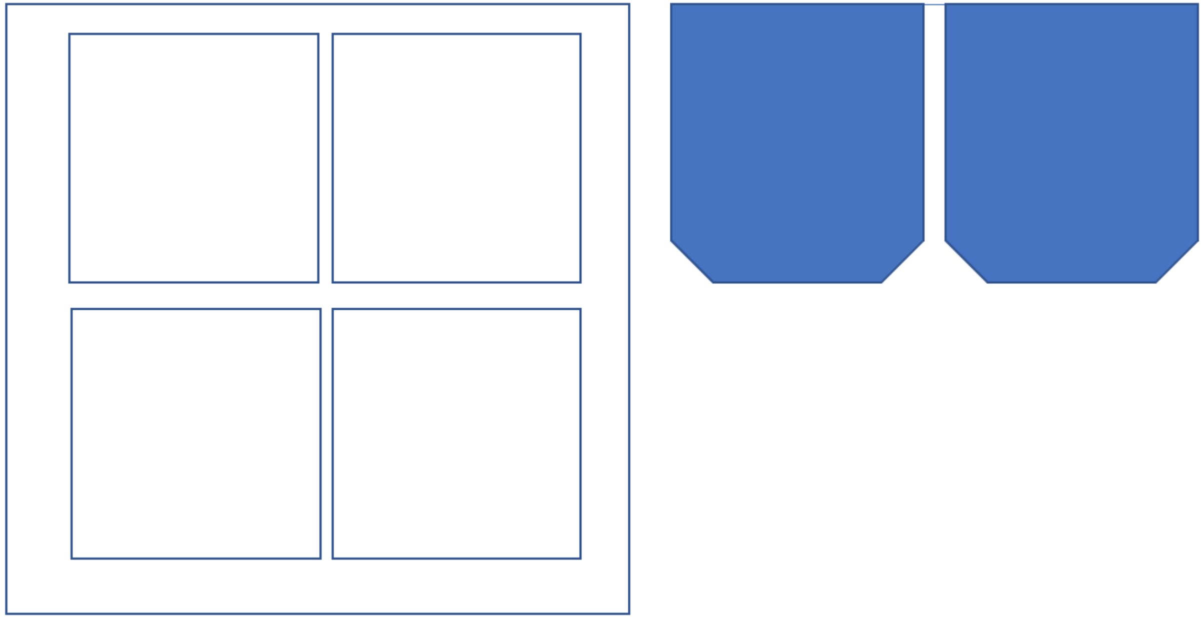
Sterile, *dnd* -mutated germ cell free salmon:

- Never become sexually mature
- Grow normally (but lack puberty-associated growth-spurt)
- Develop smaller livers (possibly due to lack of sexual maturation)
- Show normal:
  - NKA activity in gills (smoltification)
  - Prevalence of vertebra deformities
  - Heart size
  - Plasma concentration of most of the stress indicators investigated (but transiently elevated concentration of osmolality and lactate)

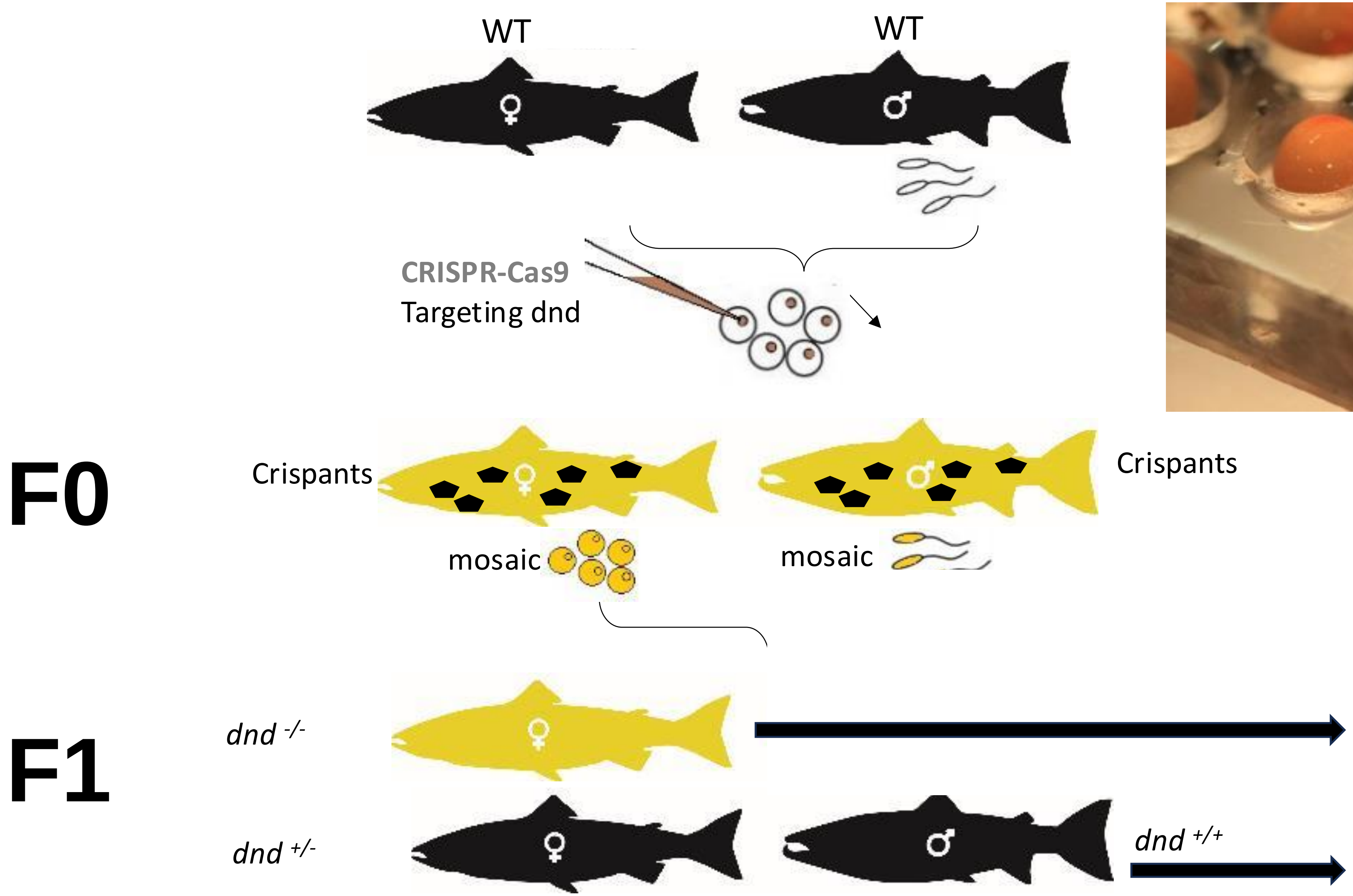
Kleppe et al 2017 Sci. Rep.
Kleppe et al 2022 Aquaculture

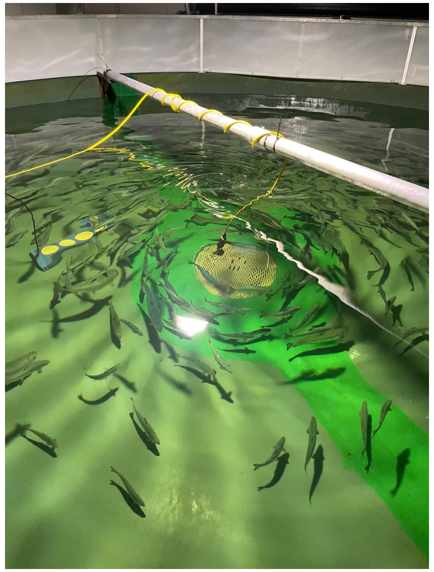
What is the welfare of genetically sterile salmon in reared in net pens



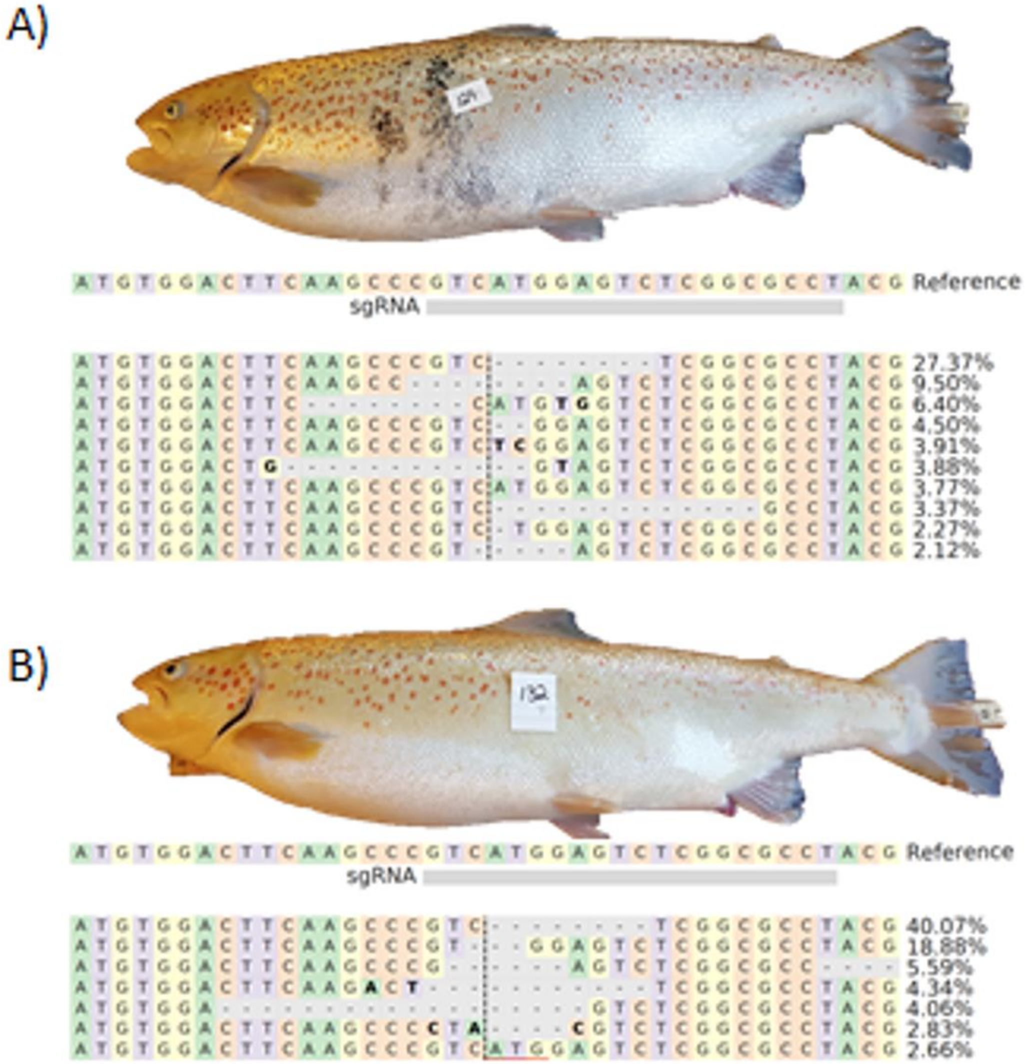


Shortened breeding scheme for long generation species

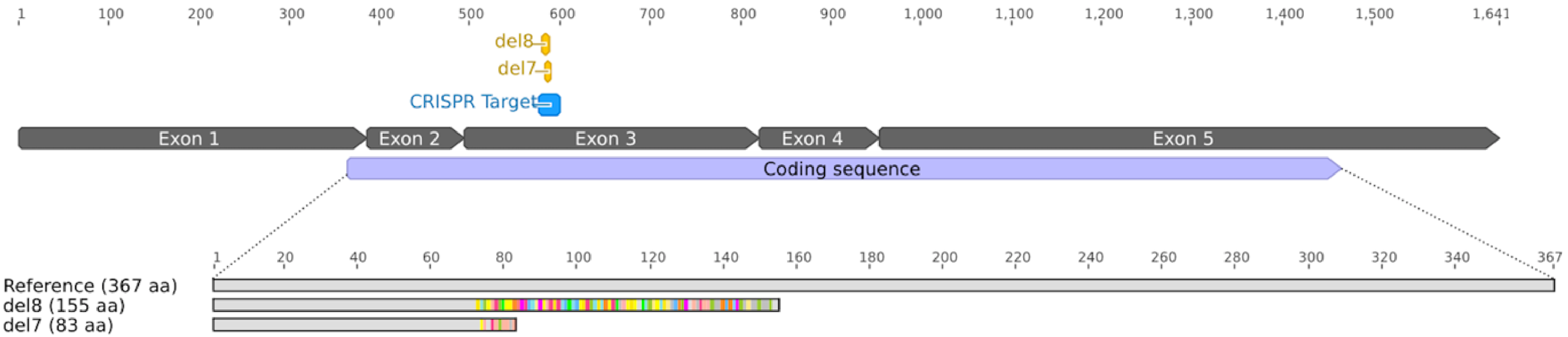


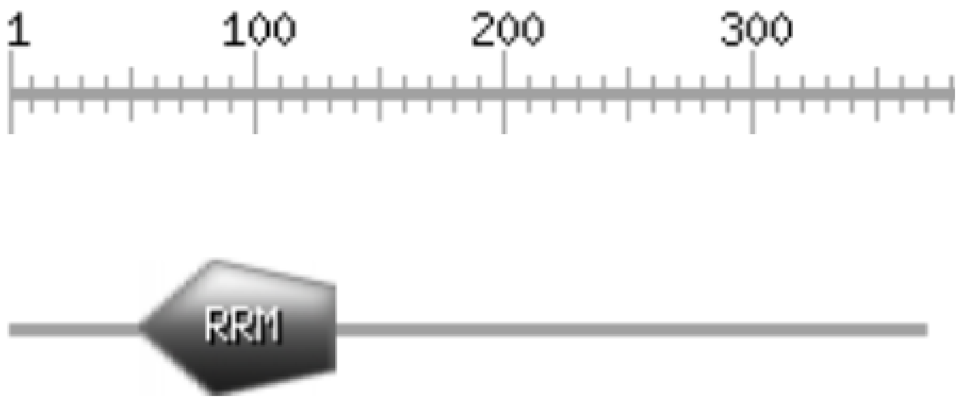


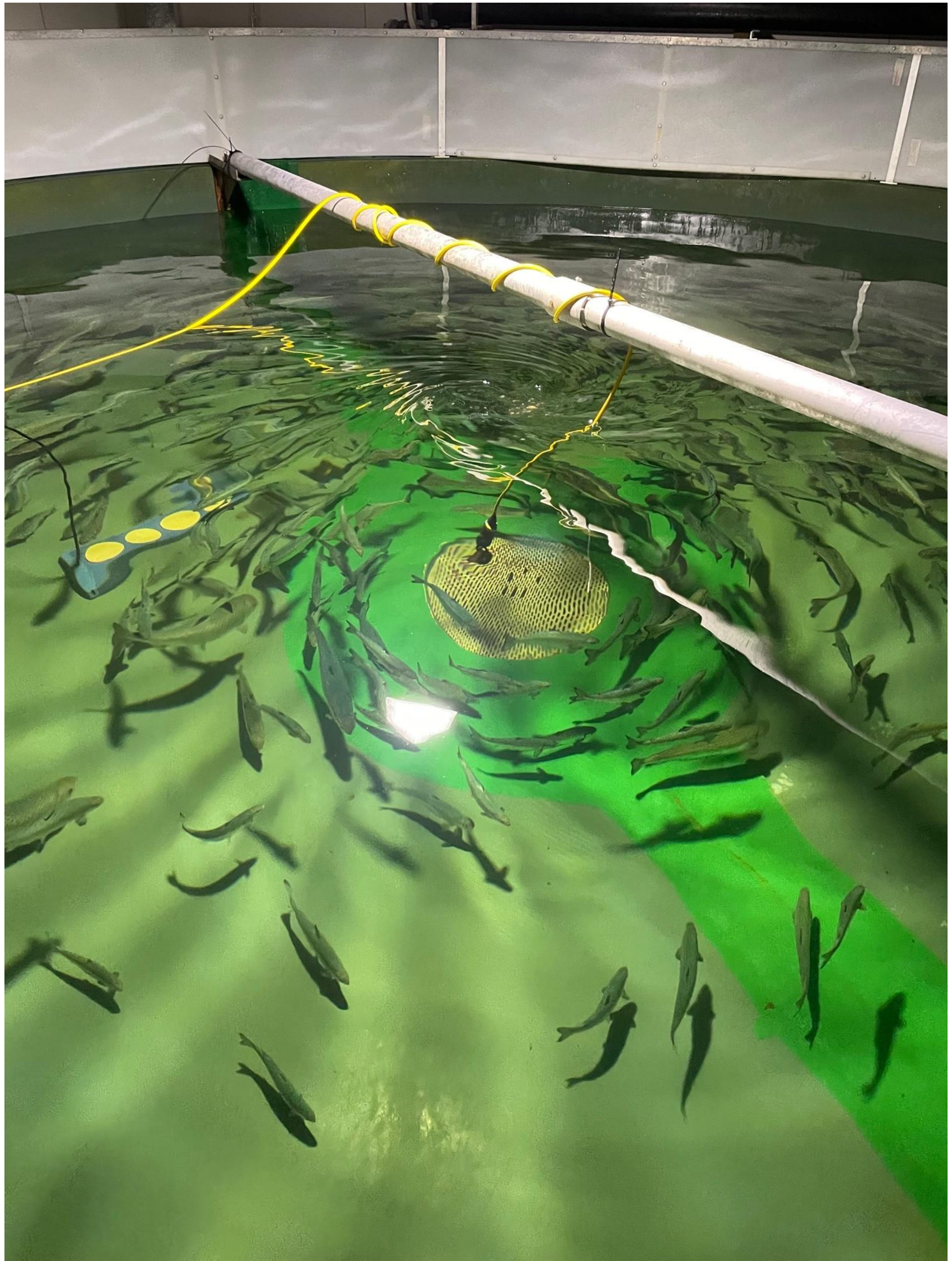
Genotypes in F0's



$dnd^{-/-}$ mutation types







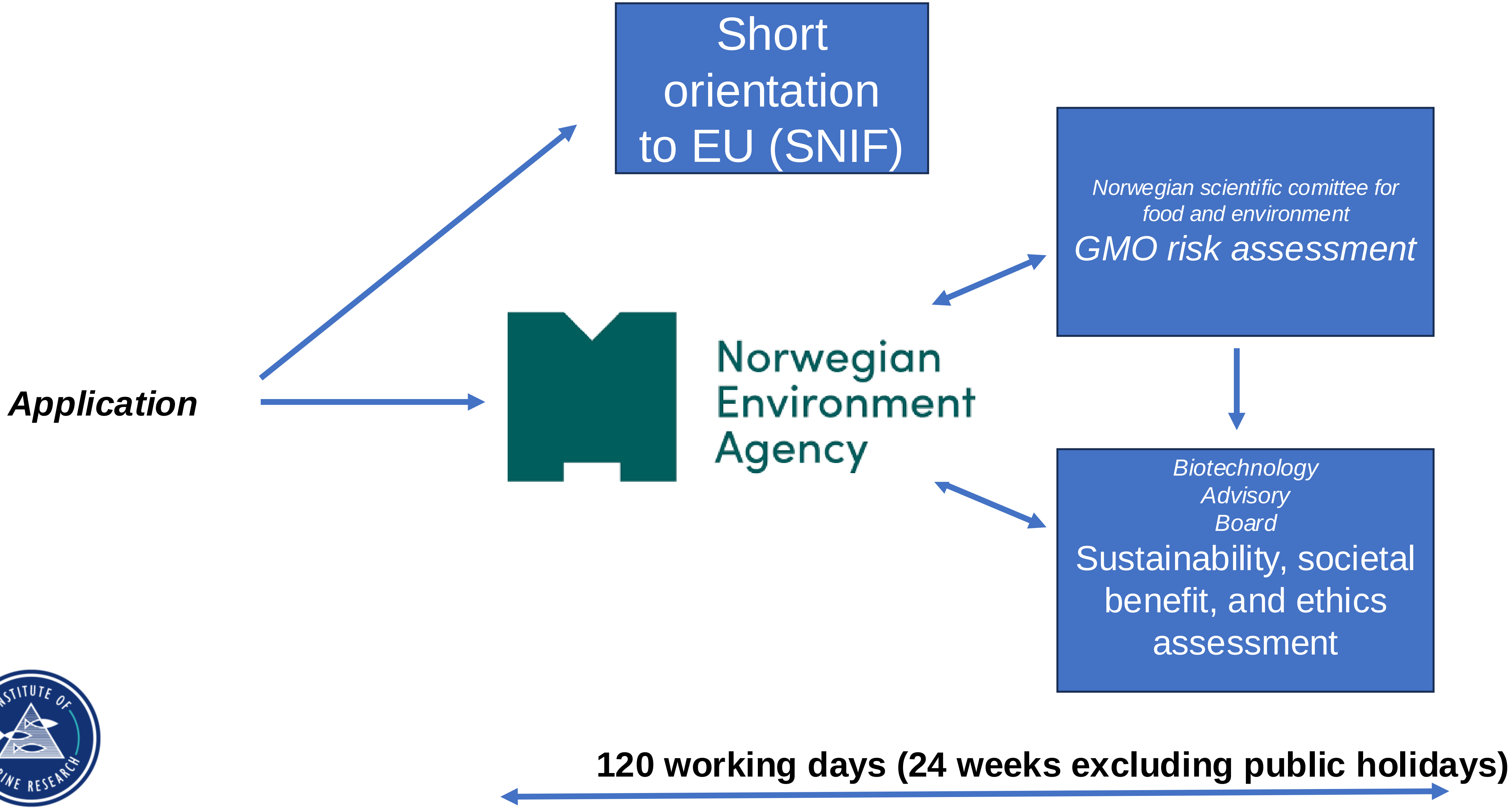
-pit tagged

-genotyped

-common garden

-reared to harvest

Application process for a Research trial in sea cages Norway



1. Submission of application, April 15th 2023 , 60 pages (Fish about 50g)

2. June-September 2023 – three round of extra data submitted

3. Some clock stops due to this

4. VKM's assessment on Oct 6 2023, day 80

The Norwegian Environment Agency commissioned this risk assessment.

Gene flow from escaped farmed salmon to wild salmon poses a problem to the wild salmon populations in Norway. To address this issue, the Institute of Marine Research has developed a salmon that is designed to be sterile, by using the genome editing technique CRISPR.

Assessment

VKM has assessed whether the field trial release of 303 genetically modified farmed salmon and 485 control salmon could have potential negative impacts on biodiversity in Norway. According to the application, the fish will be kept in sea cages, and VKM has assessed the risk they pose both in the cages and in case of their escape from these.

VKM concludes that, based on the documentation in the application, there is insufficient proof of sterility in all of the 303 genetically modified salmon and likewise, a possibility that an unknown number of the 485 fertile controls may carry a mutated allele that leads to sterility. In a worst-case scenario these alleles could be introduced to wild salmon populations, should the salmon escape from the net pens.

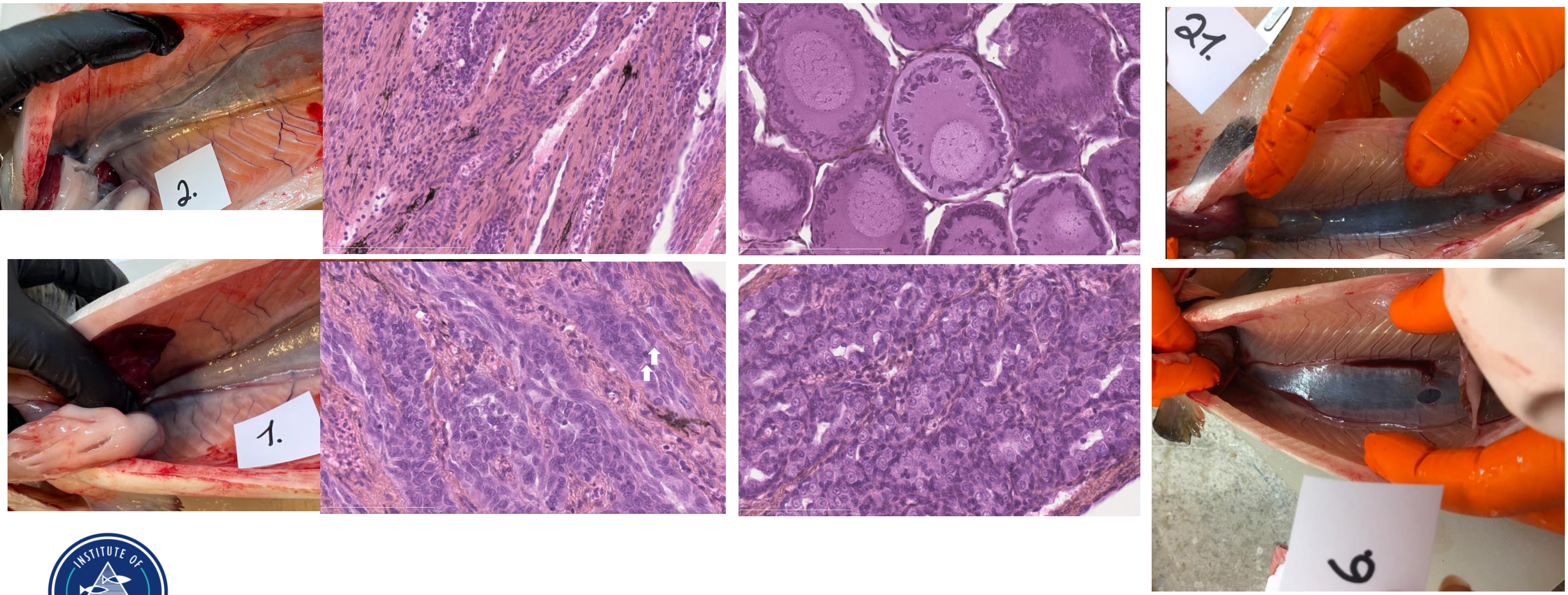
Pending approval, we submitted extra data

- Exchanged sibling controls (*wt/wt*) with production fish (n=249)
- Provided double genotyping of all *dnd*KO fish (n=185)
- Provided phenotype of all $dnd^{-/-}$ genetic variants in the tank (n=61)

Biotechnology Advisory Board
Sustainability, societal benefit, and ethics assessment

Given that the risk of genetic impact on wild salmon is avoided, the entire Biotechnology Council believes that the experimental release is suitable for generating knowledge that is beneficial to society and could contribute to promoting sustainable development.

In 15 $dnd^{-/-}$ variants no germ cells are found (n=89)



14 months after research trial submission - NO

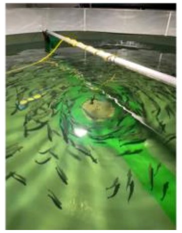
The Norwegian Environment Agency considers the pressure on wild salmon populations to be significant, and the amount of salmon returning from the sea to Norway each year has more than halved since the 1980s. Wild salmon has been listed on the Norwegian Red List of Species for 2021. It is assessed as being near-threatened. In its assessment, the Norwegian ***Environment Agency emphasizes that the Norwegian Scientific Committee for Food and Environment (VKM) has described the massive negative consequences for wild salmon populations and that VKM has presented a probability calculation indicating that a sterility allele could persist for many generations.*** *Therefore, there is no uncertainty about the massive negative consequences that the spread of sterility alleles could have for wild salmon, but the probability of this occurring has been assessed by VKM as very low.*
Based on VKM's risk assessment, the Norwegian Environment Agency therefore considers that there may be a risk of environmental damage to wild salmon stocks that are already under significant pressure.

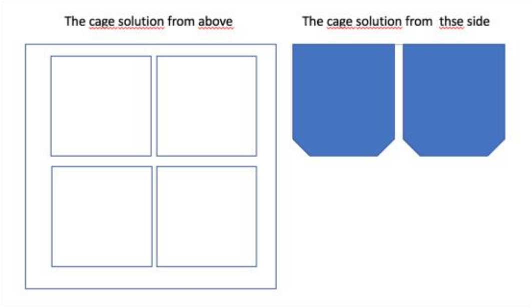
FUTURE

- Reuse sterile fish (3 kilo)
    - Surrogacy studies- shorten generation time
    - Explore whether sterile fish consume more small salmon than other salmon

- Reapply sea cage trial using a F2 generation of homozygous knockout fish

- Continue work with developing breeding for 100% sterility





Conclusions

•**Aquaculture is experiencing intensive growth, with 27,000 species of fish.**

•**Gene editing is a new tool box in breeding, vaccine development and general production**

•**Being the first to challenge older regulations is never easy.**

•**Regulatory work requires 100% more effort compared to ordinary science.**

•**In retrospect, it would have been better to use the F2 generation instead of the F1.**

•**Current risk assessments do not consider the benefits of the solution, such as saving wild salmon strains.**

•**The control fish will have 100% capability to spread farmed genes into the environment. How can this be allowed?**

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Grieg Seafood®

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Bioteknologirådet
The Norwegian Biotechnology Advisory Board

Thank your for the attention!