Genome editing in aquaculture

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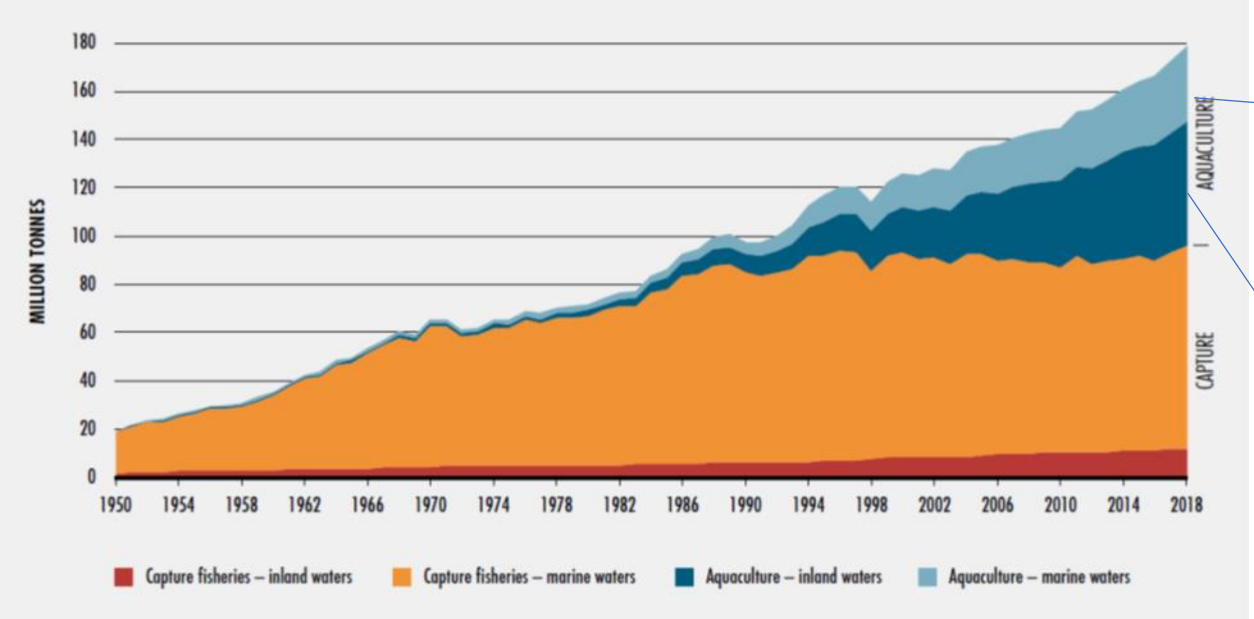
Aquaculture is important

- Aquaculture's contribution to the world supply of fisheries products has grown from 4% of world fisheries products in 1970 to over half today.
- Aquaculture products are important to human nutrition, especially in developing countries.
- Globally, aquaculture employs 20.5 million people globally, 85% of them in Asia (FAO 2020).

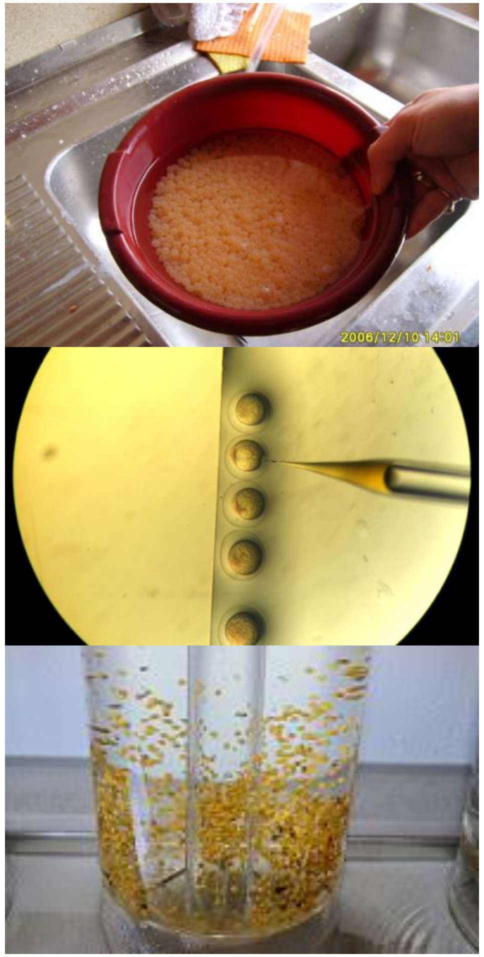


Marine

Freshwater

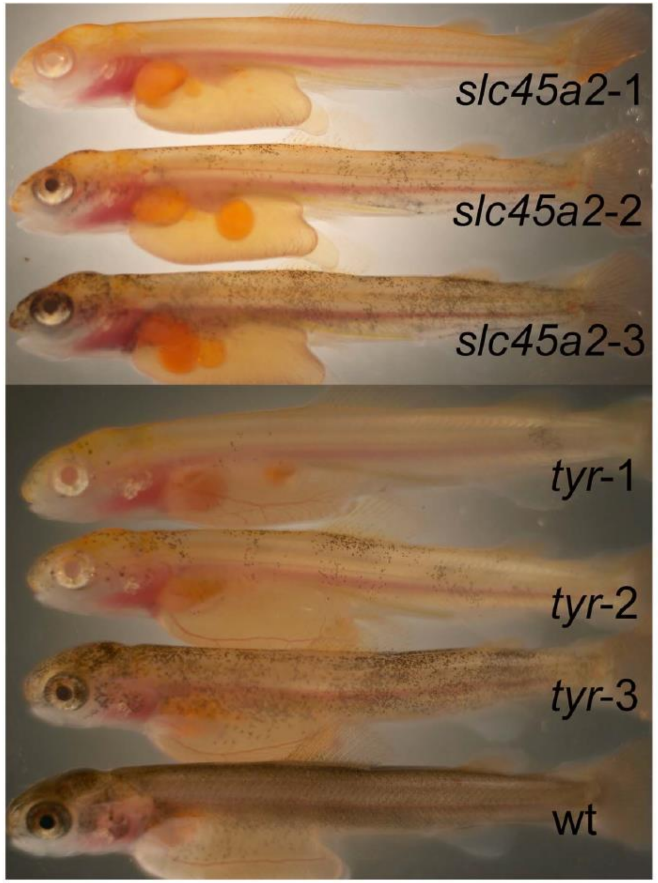
Fishes are excellent systems for genome editing

- Fishes have high fecundity.
- Protocols for artificial induction of spawning exist for many species.
- Fertilization of eggs is external, and easily conducted in vitro.
- The eggs are relatively large.
- Embryonic and larval development occur outside the mother; egg incubation and larval rearing methods are well established.
- Generation times range from one (tilapias) to several (carps, salmonids, catfishes) years.



Proof of principle and assessment of alternative approaches

- The first genome-editing experiments in fishes used model systems such as zebrafish, first using ZFNs, followed by more-efficient TALENS, and ultimately by CRISPR/Cas9.
- Successful protocols then adapted to aquaculture species, often using marker genes to readily show transformation, for example…
  - Edvardsen et al. (2014) selected the solute carrier family 45, member 2 (*slc45a2*) and tyrosinase (*tyr*) genes known to be involved in pigmentation in fishes as marker genes.
  - 40 and 22% of injected Atlantic salmon embryos showed mutations at *slc45a2* and *tyr*, respectively.
  - The mutations displayed a range of phenotypes ranging from complete lack of pigmentation, to partial loss, to normal pigmentation.
  - → CRISPR/Cas9 *can* induce double-allelic knockout in the F_0 generation, *but* be cognizant of the possibility of mosaicism.

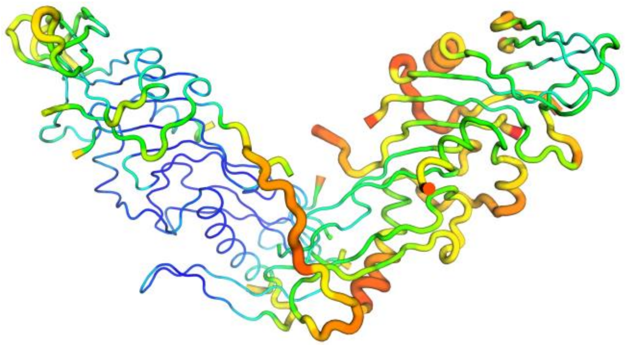


Subsequently, many cultured species have been genome-edited for valued traits…

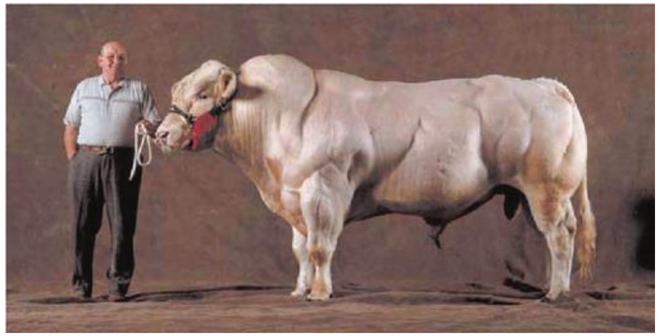
Species or group	Share of 2018 production	Growth, muscle development	Reproductive confinement	Disease resistance	Other trait
Grass carp	10.5			X	
Silver carp	8.8				
Nile tilapia	8.3	X	X		
Common carp	7.7	X			
Bighead carp	5.8				
Catla	5.6				
Crucian carps	5.1				Coloration
Atlantic salmon	4.5		X		Coloration, fatty acid metabolism
Striped catfish	4.3				
Rohu,	3.7			X	
Milkfish	2.4				
Torpedo-shaped catfishes	2.3				
Rainbow trout	1.6	X			
Wuchang bream	1.4				
Black carp	1.3				
Yellow catfish	0.9	X			
Channel catfish	-	X	X	X	
Large-scale loach	-				Coloration
Olive flounder	-	X			
Pacific bluefin tuna	-				Swimming behavior
Pacific oyster	-				Myosin function
Red sea bream	-	X			
Ridgetail white prawn	-				Chitinase function
Southern catfish	-		X		
Tiger pufferfish	-	X			

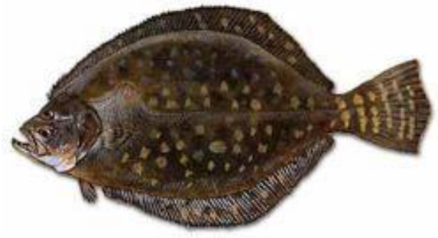
- *… How has genome editing been applied to traits valued in aquaculture?*

Enhancement of growth and muscle development



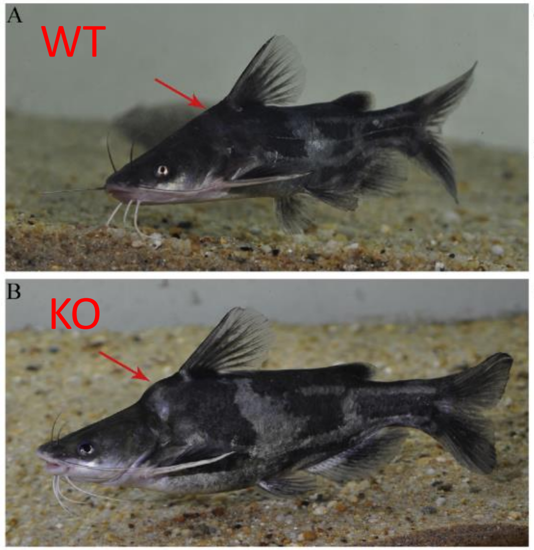
- Common breeding goals!
- <u>Myostatin</u>: A key regulator of skeletal muscle growth in all vertebrates.
- Expression of *mstn* is linked with the double-muscled phenotype in Belgian Blue and Piedmontese cattle, as well as other animals, resulting in increased muscling compared to breeds that lack the causal mutation.
- *Mstn* knockout led to increased muscle mass in mice.
- Since then, applied to:
  - Common carp
  - Channel catfish
  - Olive flounder
  - Red sea bream
  - Yellow catfish
  - Nile tilapia

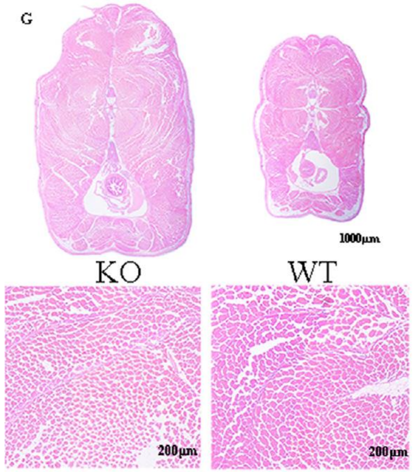




Enhancement of growth and muscular development

- Yellow catfish is an important aquaculture species in China, but small size and low fillet yield limit its value.
- Dong et al. (2011) disrupted the *mstna* gene using ZFNs:
  - Homozygous mutants displayed double-muscling, with two obvious muscle masses between the head and dorsal fin at 1-month-old, which become more obvious with growth.
  - Body weight of *mstna*-null fish was 1.27-1.37-fold higher than their wild-type siblings at 80 and 210 dpf.
  - Histological analysis → *mstna*-knockout fish had increased numbers of fibers, with decreased fiber size.
- Zhang et al. (2020) → genome-edited *mstna* fish grew and bred normally.



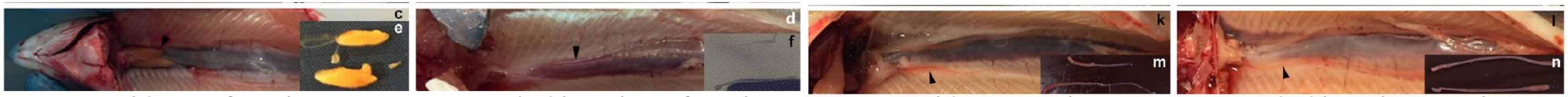


Reproductive confinement

- Cultured fishes escape from aquaculture facilities, where:
  - they pose ecological impacts upon receiving ecosystems, especially if they are a non-native species, and
  - they pose genetic impacts upon populations with which they can interbreed.
- Reproductive confinement protects the interest of the breeder in their investment in a genetic improvement program.
- Hence, lines of research have been opened into reproductive confinement of several key species:
  - Nile tilapia
  - Channel catfish
  - Atlantic salmon…

Reproductive confinement

- Wargelius et al. (2016) sought to produce germ cell-free Atlantic salmon by knocking out *dnd*, which encodes a factor required for survival of germ cells.
- Induced *dnd* mutations produced fish lacking germ cells.
- Kleppe et al. (2017) → germ cell-free fish salmon remained immature and did not undergo puberty.



Wild-type female | *dnd*-knockout female | Wild-type male | *dnd*-knockout male

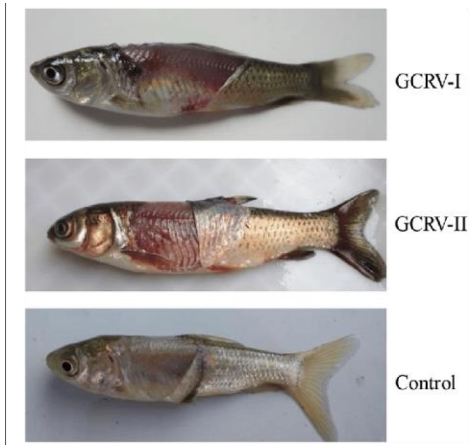
- Salmon lacking germ cells cannot be used for breeding; hence, a strategy for *recovering* reproductive ability is needed for these fish to prove useful for aquaculture.
- Guralp et al. (2020) reported a rescue approach for producing germ cells in *dnd* knockout fish:
  - Co-injected the wild-type variant of *dnd* mRNA together with the CRISPR/Cas9 constructs targeting *dnd* into one-cell stage embryos.
  - Rescued one-year-old fish contained germ cells, type A spermatogonia in males and previtellogenic primary oocytes in females.
- Demonstration of rescue opens the possibility for large-scale production of germ cell-free Atlantic salmon offspring.

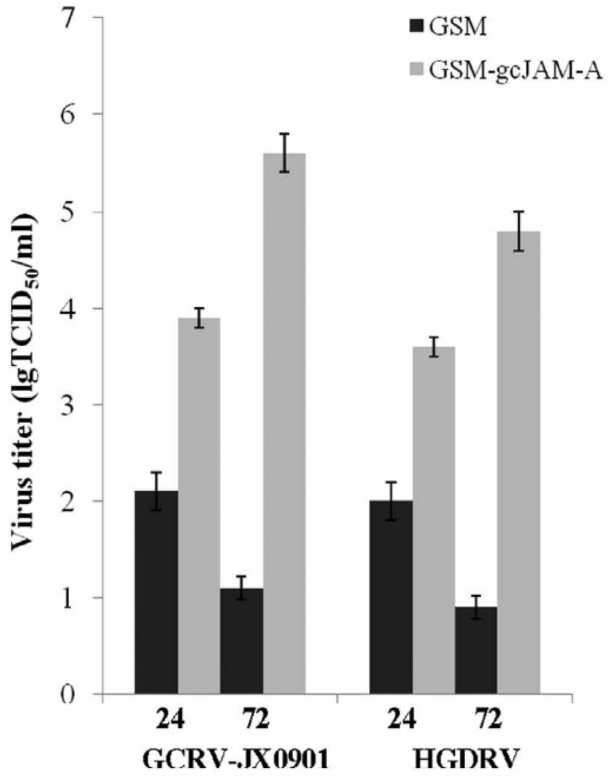
Disease resistance

- Aquaculture stocks are held at high population densities, and fish may be subject to physiological or social stress, rendering them susceptible to parasites and pathogens.
- Loss to disease is a *major* threat to aquaculture enterprises…
- Genetic improvement of disease resistance is a high breeding priority.
- Several genome-editing experiments have addressed improvement of disease resistance:
  - Rohu
  - Grass carp
  - Channel catfish

Disease resistance

- Hemorrhagic disease of grass carp is caused by grass carp reovirus (GCRV), which has many genotypes, and leads to huge economic losses.
- Junctional adhesion molecule-A (*JAM-A*), a member of the immunoglobulin superfamily, may be useful for developing therapies against GCRV infection.
- Ma et al. (2018) knocked out the grass carp *JAM-A* gene and evaluated *in-vitro* resistance against various GCRV genotypes:
  - CRISPR/Cas9 effectively knocked out *JAM-A* and reduced infection for two different GCRV genotypes in grass carp kidney cells.
  - The results showed that JAM-A is necessary for GCRV infection, and suggested knockout as an approach for control of the disease.





Other breeding goals

- Genome editing can be applied to understand genes affecting valued traits, and ultimately to approach other breeding goals:
  - Muscle contraction properties – Pacific bluefin tuna
  - Coloration – large-scale loach, white crucian carp, Atlantic salmon
  - Fatty acid composition of flesh – Atlantic salmon

Other aquaculture species

- The scope of world aquaculture includes mollusks, crustaceans, and seaweeds; recent work has developed protocols for genome editing of these taxa:
  - Mollusks: Yu et al. (2019) delivered CRISPR/Cas9 ribonucleoproteins into Pacific oyster eggs.
  - Crustaceans: are difficult to transform ← fertilization is internal. Gui et al. (2016) applied CRISPR/Cas9 technology, targeting the chitinase 4 *EcChi4* gene of ridgetail white prawn.
  - Seaweeds: the largest sector of world aquaculture – no reports of genome editing of seaweeds.

Genome editing provides powerful tools for genetic improvement of aquaculture stocks…

- *What will it take to achieve practical adoption of genome-edited fishes?*
- Industry must see a pathway to commercialization, with:
  - Risk-scaled, enabling regulatory policy,
  - Use of well-confined culture systems,
  - Measures to promote consumer acceptance.

On towards commercialization?

- The genome-edited Nile tilapia FLT-01 line developed by AquaBounty:
  - Has a homozygous 26-bp deletion → early stop codon in the myostatin gene.
  - Loss-of-function increases muscle mass, fish weight, and fillet yield.
  - Line was created using microinjection with nuclease mRNA; no introduction of DNA was involved. There are no off-target sites of modification.
- The fish is potentially ready for production.
  - Because there is no new genetic material or unwanted integration of plasmid DNA in the final product, it is not covered by the definition of a regulated article under Res. 763 under the Cartagena Protocol.
  - Under Argentine Resolution 173/15 – New Breeding Techniques, this fish is *not* a GMO.
  - Brazil made a similar determination in 2019.
- *This may become the first genome-edited animal product to reach the market*.

Take-home messages

- Aquaculture is important to human nutrition, especially in Asia.
- Fishes are amenable to genome editing, which has been applied to improve:
  - growth of muscle,
  - control of reproduction,
  - resistance to disease, and
  - other traits.
- Genome-edited fish (e.g., FLT01 Nile tilapia) can be produced in commercial aquaculture with:
  - risk-scaled, enabling regulatory policy,
  - use of well-confined culture systems,
  - measures to promote consumer acceptance.

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Thank you