Overview and applications of biotechnology in aquaculture and fisheries for food, agriculture and climate change resiliency

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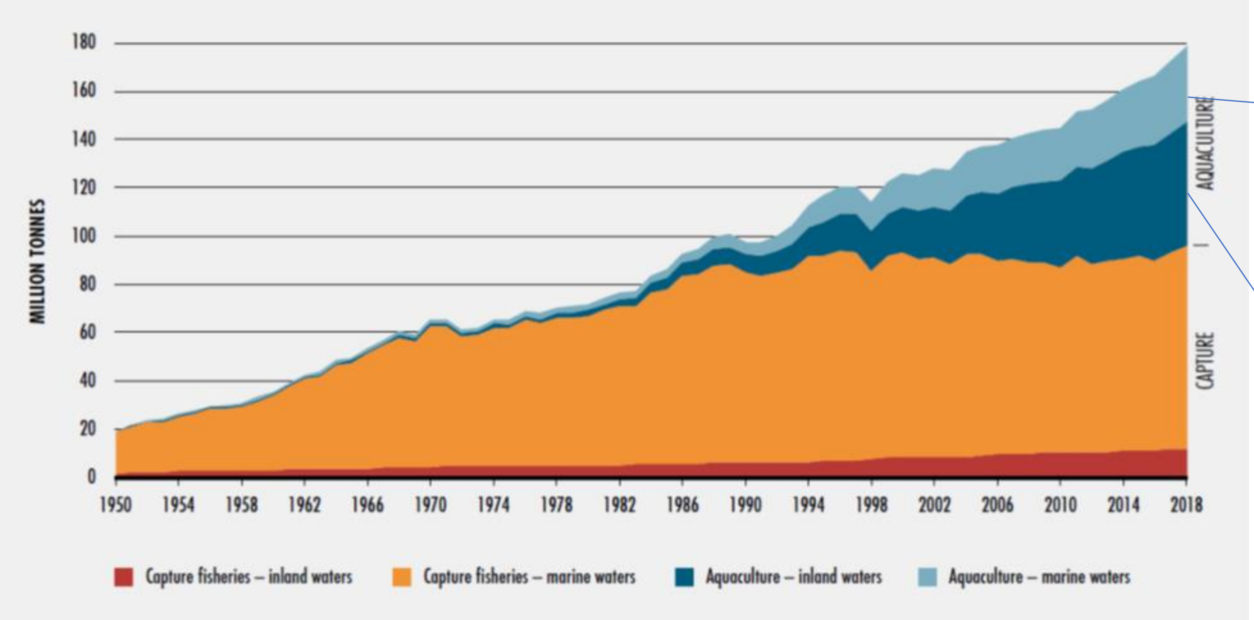
Aquaculture is important

- 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 economies.
- Globally, aquaculture employs 20.5 million people, 85% of them in Asia (FAO 2020).



Marine

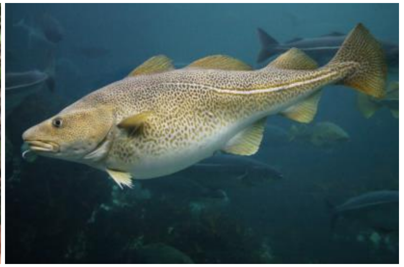
Freshwater

A diverse sector, with many species

- Over 600 cultured species identified by UNFAO, although…
- 15 species contribute over 74% of world production
- These species vary from tropical to subpolar species:


Nile tilapia


Pacific white shrimp


Atlantic cod

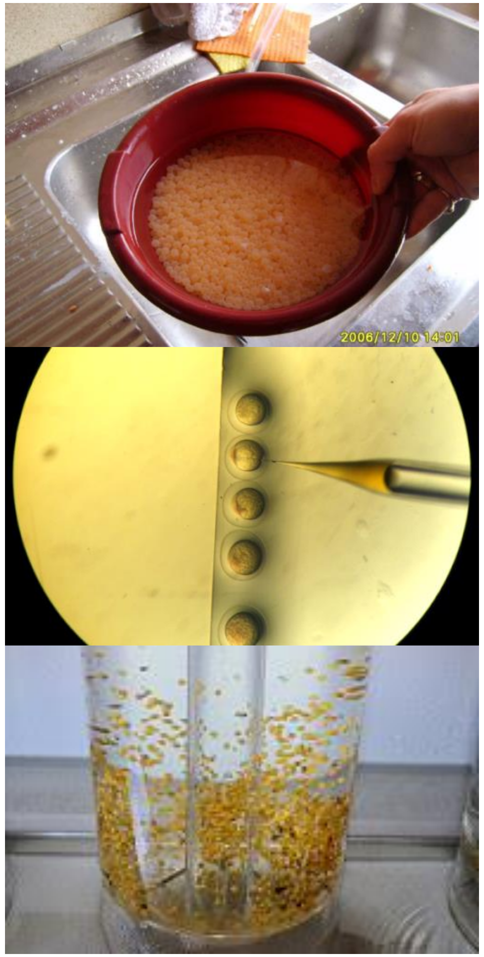

Atlantic salmon

- *The aquaculture sector has opportunities to achieve climate-change resiliency simply by making informed choices of suitable species.*
- But what can we do to foster improvement *within* species?
- Some scope for selective breeding, and…

Species or group	Share of 2018 production
Grass carp	10.5
Silver carp	8.8
Nile tilapia	8.3
Common carp	7.7
Bighead carp	5.8
Catla	5.6
Crucian carps	5.1
Atlantic salmon	4.5
Striped catfish	4.3
Rohu	3.7
Milkfish	2.4
Torpedo-shaped catfishes	2.3
Rainbow trout	1.6
Wuchang bream	1.4
Black carp	1.3
Yellow catfish	0.9

Fishes are excellent systems for gene transfer and genome editing

- Fishes have high fecundity.
- Protocols for artificial induction of spawning exist for cultured species.
- The eggs are relatively large, fertilization is external and easily conducted *in vitro*.
- 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.
- Many fish species have been modified for valued traits…



Many cultured fishes *have* been genome-edited for valued traits…

Species or group	Growth, muscle development	Reproductive confinement	Disease resistance	Other trait
Grass carp			X	
Silver carp				
Nile tilapia	X	X		
Common carp	X			
Bighead carp				
Catla				
Crucian carps				Coloration
Atlantic salmon		X		Coloration, fatty acid metabolism
Striped catfish				
Rohu			X	
Milkfish				
Torpedo-shaped catfishes				
Rainbow trout	X			
Wuchang bream				
Black carp				
Yellow catfish	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			

Other aquaculture species

The scope of world aquaculture also includes mollusks, crustaceans, and seaweeds

Recent work has developed protocols for genome editing of these taxa:

Mollusks: Eggs are tiny and are broadcast.

- Yu et al. (2019) delivered CRISPR/Cas9 ribonucleoproteins into Pacific oyster eggs.

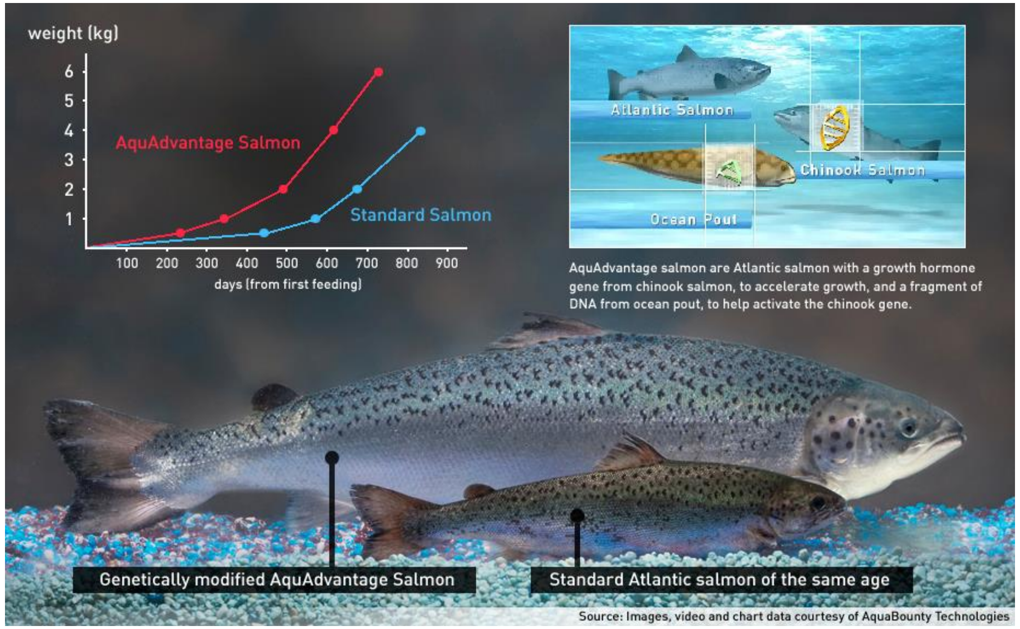
Crustaceans: Fertilization is internal, so difficult to transform.

- 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, with many, varied species.

- No reports of genome editing of seaweeds.

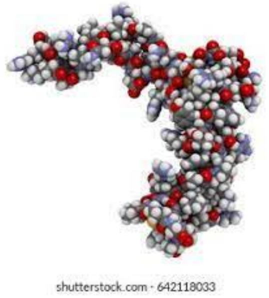
Modifications for growth and muscle development



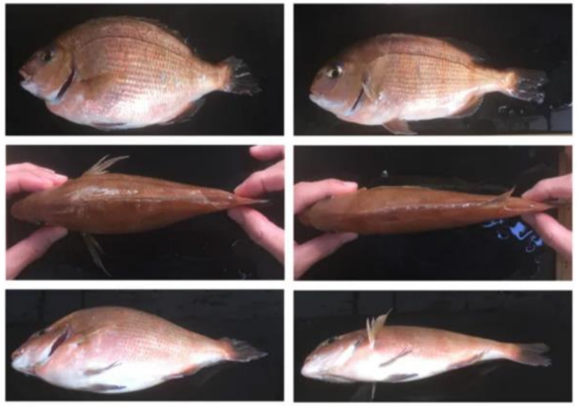
Classical gene transfer

- Growth hormone gene → Nile tilapia, channel catfish, carps, loach, many other species, including…
- AquaBounty AquAdvantage Atlantic salmon; op*AFP*-cs*GH* → rapid growth rate
- Production time is roughly halved and feed conversion efficiency is increased ~10%
- Approved for commercial production in Canada and the United States, pending in other countries

Modifications for growth and muscle development



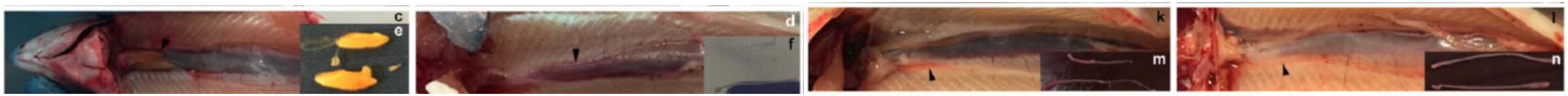
- Genome editing
  - Knockout of myostatin gene → increased muscle growth
  - Applied to Nile tilapia, common carp, rainbow trout, yellow catfish, channel catfish, olive flounder…
  - Regional Fish Institute (Japan) → Red sea bream and tiger pufferfish
  - Approved for production in Japan, where not regarded as a GMO



Modifications for promoting sustainability

Genome editing

- Reproductive confinement of Nile tilapia, channel catfish, southern catfish, and Atlantic salmon
- Escapees from aquaculture could not interbreed with locally-adapted wild populations
- Wargelius group (Norwegian Institute for Marine Research) →proof-of-principle for both sterilization of Atlantic salmon for production, rescue of broodstock candidates.



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

Modification to address resiliency to climate change

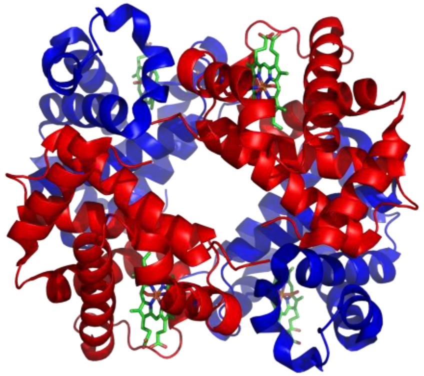
<u>Upper temperature tolerance</u>

- Classical selective breeding:
  - Upper thermal tolerance (UTT) *is* a heritable trait
  - Heritability for UTT varies among and within species: Atlantic salmon – 0.47 (Benfey et al 2022), Rainbow trout – 0.41±0.07 (Perry et al. 2005), Turbot – 0.087±0.032 (Zhang et al 2014), 0.111±0.080 (Ma et al. 018), 0.247±0.108 (Wang et al. 2019)
  - Rainbow trout showed response to selection for UTT (Chen et al. 2015)
- Molecular marker-assisted breeding:
  - We have some knowledge of QTLs for thermal tolerance in rainbow trout (Jackson et al. 1998, Perry et al. 2001) and Arctic charr (Somorjai et al. 2003) – but knowledge of QTLs is far short of knowledge of the causal gene variants
  - There may be little potential for response to selection in tropical fishes (Morgan et al. 2020).
- We need well-defined molecular targets for gene transfer or genome editing
  - Perhaps heat-shock proteins?
  - Known to be physiologically important in fishes and mollusks
- *We need more and well-targeted, fundamental research!*

Modification to address resiliency to climate change?

Availability of dissolved oxygen

- Dissolved oxygen reaches saturation in the ppm range, often 6-10 ppm
- Oxygen is less soluble in warm than in cold water
- Hemoglobin (Hb) of aquatic species has a more critical tradeoff between oxygen uptake and delivery than does Hb of terrestrial species
- Might we prove able to edit Hb of aquatic species to favor oxygen uptake (while retaining the Root effect for fishes to fill the gas bladder)?
- *We need targeted, fundamental research!*
- I expect greater emphasis on the agrotechnical side, using low-tech approaches to oxygenate culture systems.



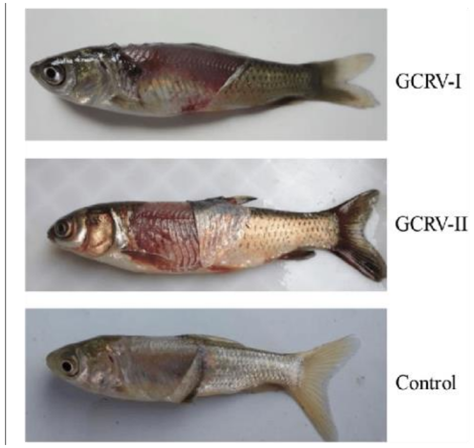
Modification to address disease resistance

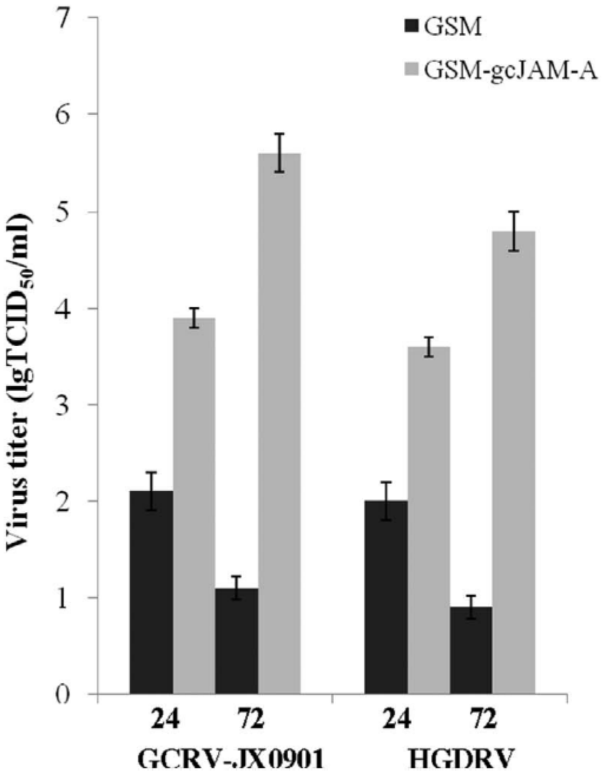
- Aquaculture stocks are already held at high population densities, subjecting stocks to physiological or social stress, rendering them susceptible to parasites and pathogens.
- Heightened temperature will *increase* their susceptibility to disease outbreaks.
- Loss to disease is a major threat to aquaculture enterprises…
- Genetic improvement of disease resistance is a high breeding priority.
- Several genome-editing experiments have addressed improvement of disease resistance:
  - Channel catfish
  - Rohu
  - Grass carp …



Disease resistance

- Hemorrhagic disease of grass carp is caused by grass carp reovirus (GCRV), which has many genotypes, and leads to huge economic losses.
- The junctional adhesion molecule-A (*JAM-A*) is a member of the immunoglobulin gene superfamily.
- 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.
- *I expect much more effort on using genome editing to improve disease resistance traits*.





Role of selective breeding and biotechnology for genetic improvement – my predictions

- Climate change will *complicate* production in low-tech systems in tropical areas – *where resiliency is already an issue*.
- Classical and molecular marker-assisted breeding will contribute to production resiliency, as will improved agrotechnical practices.
- Biotechnology can make *unique* contributions that classical breeding cannot, e.g., heightened disease resistance.
- *For biotechnology to contribute to its fullest potential, we will need:*
    - More fundamental research into the molecular mechanisms of underlying key traits
    - Risk-scaled enabling public policies
    - Public understanding and acceptance

Applications of biotechnology for food, agriculture, and climate change resiliency

Thank you