Breeding systems and biotechnology in aquaculture

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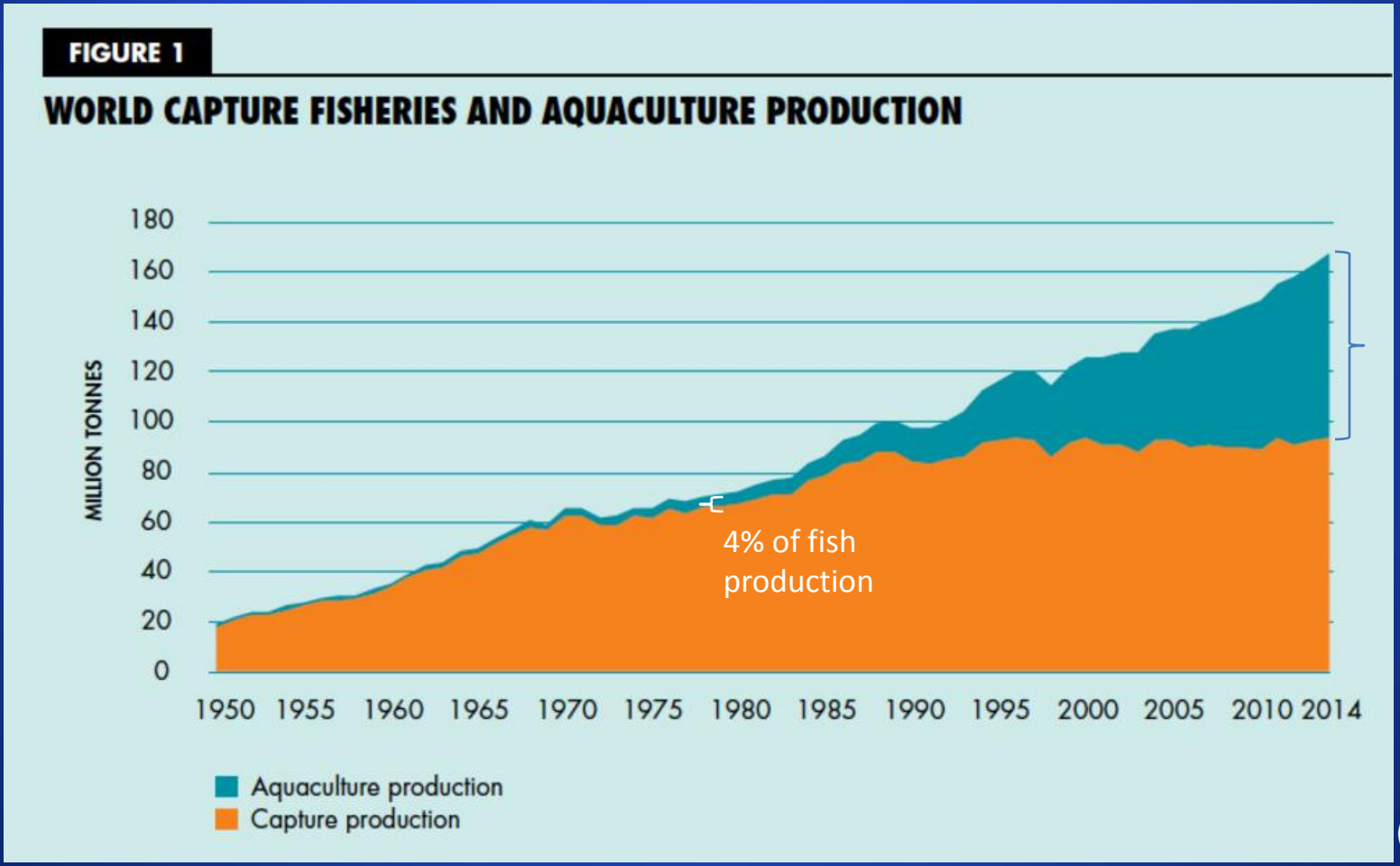
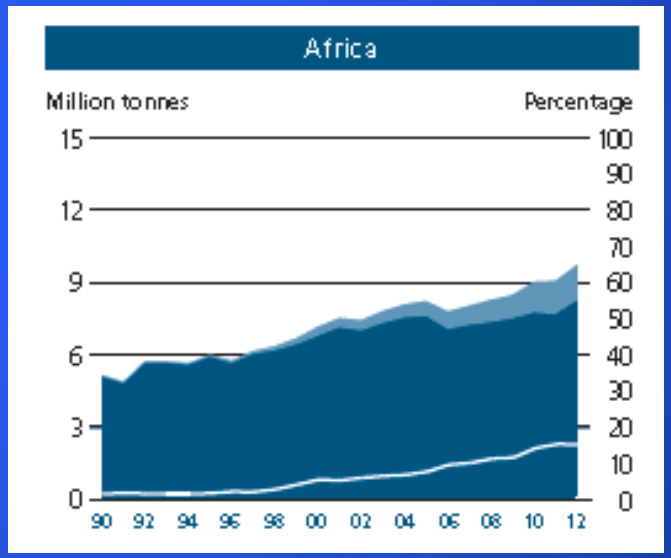
Fisheries is the last major food system where harvest from the wild remains important, …and aquaculture provides a large and growing part of our supply of fisheries products



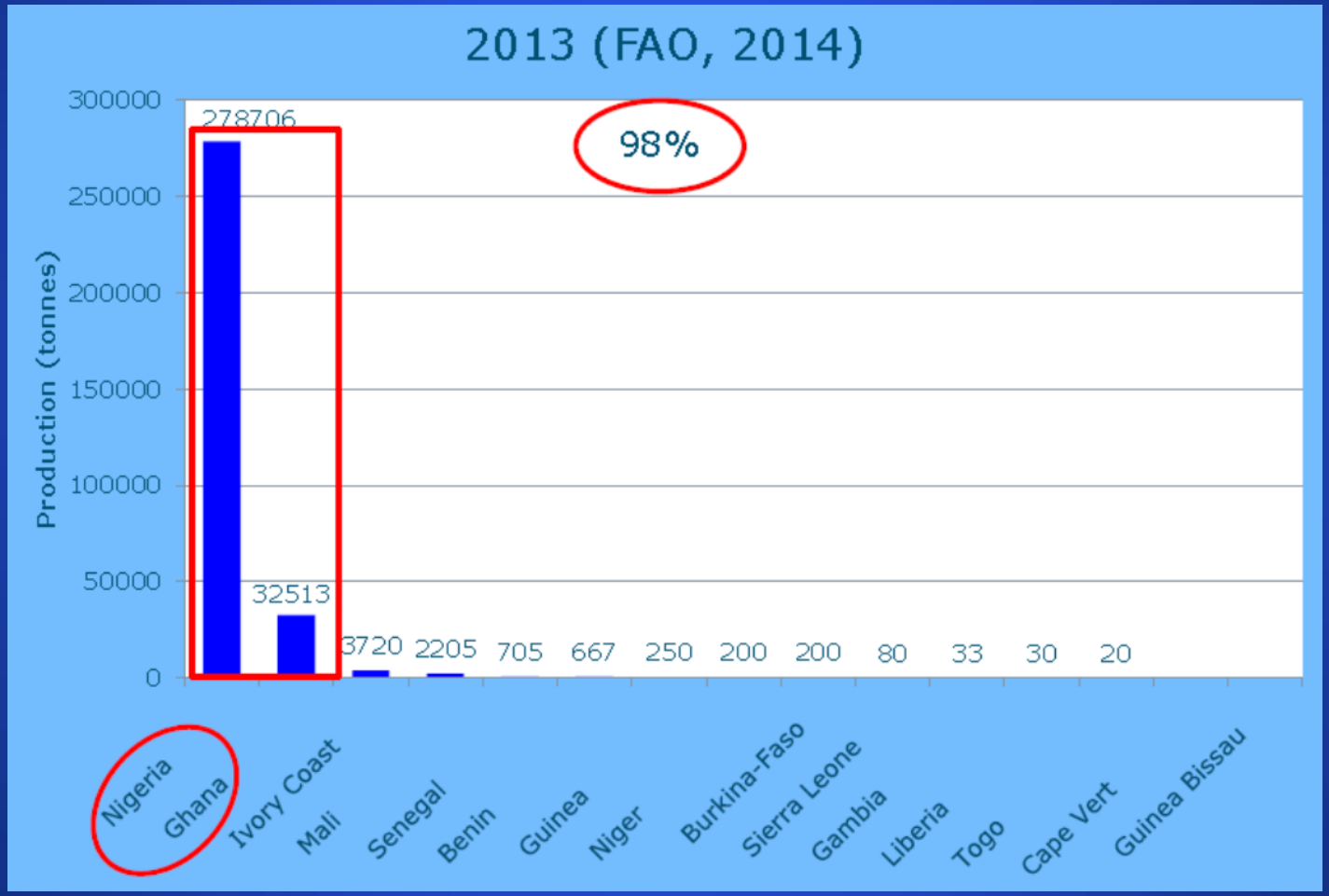
(FAO 2014)

Aquaculture production

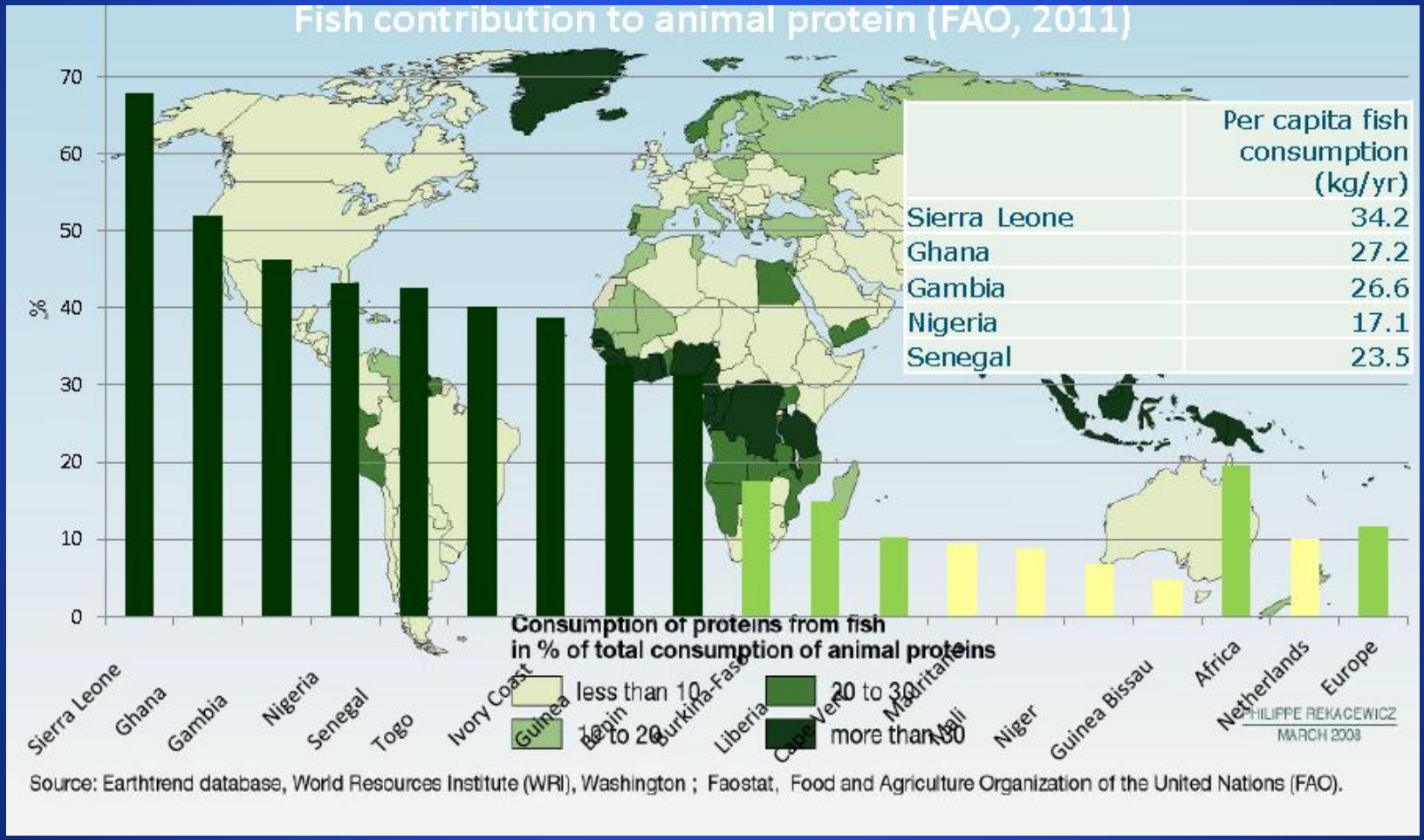
- Huge in Asia – 55% of fish production (FAO 2014)
- Not huge in Africa – 18%, but growing at 10% per year
- Key species:
  - Nile tilapia
  - African catfish



Aquaculture production in West Africa



Fish consumption in West Africa



Source: Earthtrend database, World Resources Institute (WRI), Washington ; Faostat, Food and Agriculture Organization of the United Nations (FAO).

Aquaculture is a rapidly evolving sector

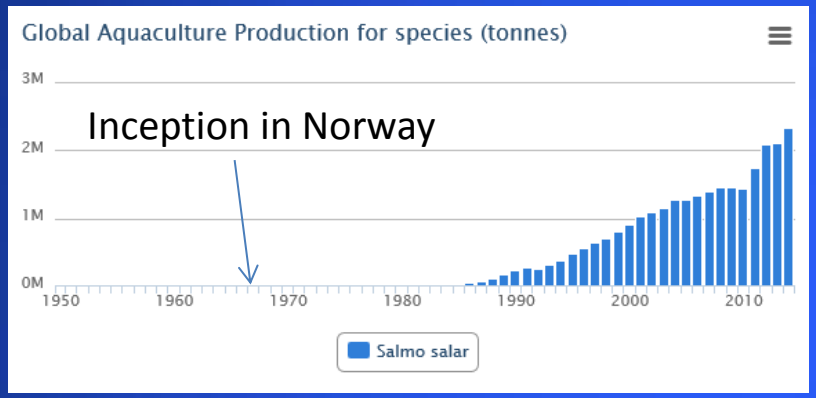
- Growing rapidly – 5.8% per annum in 2014 (FAO)
- Some sectors are “mature”, others are still emerging
- New species are coming into culture; culture systems are still being developed; some cultured species are still being domesticated
- Genetically improved lines are at very different stages of development among sectors and regions
- Distribution of genetically improved lines reflects the structure of each sector (reflects sector “maturity”, vertically integrated corporation vs. family-scale farms)

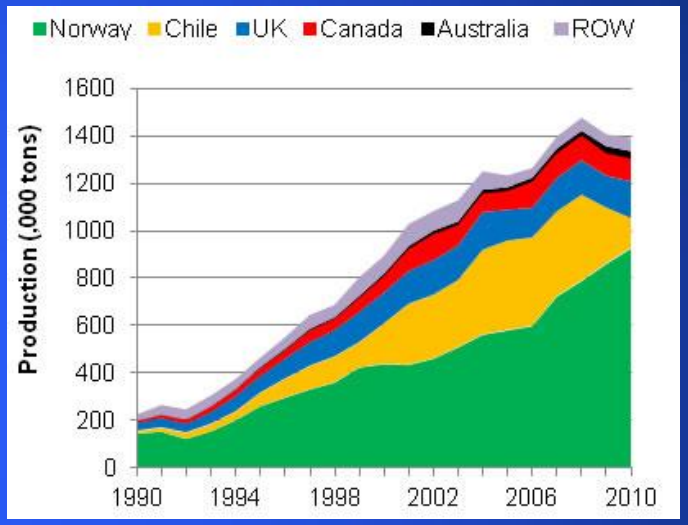
Case studies of selective breeding in key sectors of aquaculture

- World production
- Culture systems
- Industry structure
- Development and distribution of genetically improved lines
- Prospects for GE lines

Atlantic salmon

- *Salmo salar*





- Classical production system:

Atlantic salmon

- Virtually *all* Atlantic salmon in production is genetically improved
- An *exceptional* situation within aquaculture
- *Why is that?*

Industry structure:

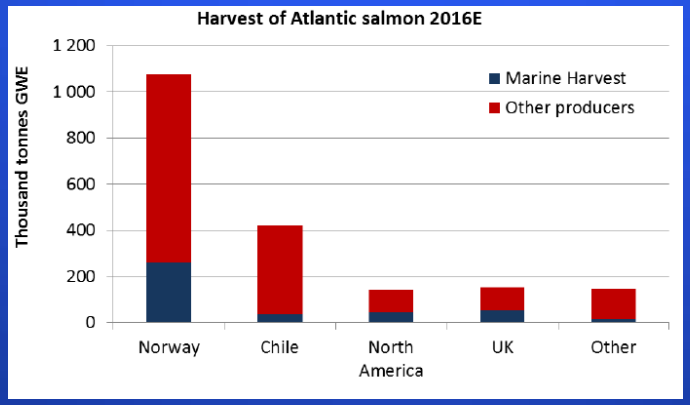
- Dominated by a few producers within each country:

	Top 10 Norway	Harvest	Top 5 UK [1]	Harvest	Top 5 North America [1]	Harvest	Top 10 Chile	Harvest
1	Marine Harvest	254 800	Marine Harvest	50 100	Cooke Aquaculture	42 000	Empresas Aquachile	63 000
2	Salmar	136 400	Scottish Seafarms	27 000	Marine Harvest	40 100	Marine Harvest	62 500
3	Lerøy Seafood	135 000	The Scottish Salmon Co.	25 600	Mitsubishi (Cermaq)	21 000	Mitsubishi	60 000
4	Mitsubishi (Cermaq)	58 000	Cooke Aquaculture	19 000	Grieg Seafood	14 300	Salmones Multiexport	51 000
5	Nordlaks	39 000	Grieg Seafood	16 400	Northern Harvest	13 000	Camanchaca	39 000
6	Nova Sea	37 400					Australis Seafood	38 100
7	Midt-Norsk / Bjørøya	32 000					Pesquera Los Fiordos	30 000
8	Grieg Seafood	31 700					Blumar	25 800
9	Norway Royal Salmon	27 900					Cooke Aquaculture	25 000
10	Alsaker Fjordbruk	27 000					Ventisqueros	22 000
	Top10	779 200	Top 5	138 100	Top 5	130 400	Top 10	416 400
	Total	1 110 800	Total	149 700	Total	139 900	Total	531 800
	Total	70 %	Total	92 %	Total	93 %	Total	78 %

Note: All figures in tonnes GWE for 2015

1) UK and North American industry are best described by top 5 producers.

- Some producers are multinational companies (Marine Harvest, Cooke Aquaculture, Mitsubishi, …)



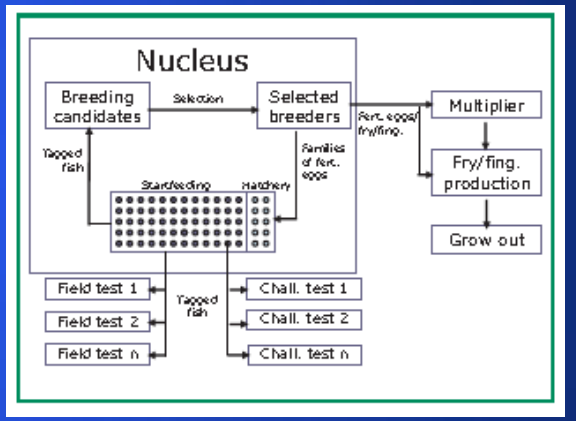
e.g., Marine Harvest has operations on three continents

Atlantic salmon

- Substantial investment in development and distribution of genetically improved lines:
- Since ~1970, Norwegian national program
- Classical selective breeding for growth rate, FCE, carcass quality, disease resistance
- Spun off to private sector
- Today, ten competing companies and organizations selectively breed Atlantic salmon. Implications:
- → more secrecy, little reporting in scientific literature…
- → substantial resources for selective breeding
- → vertical integration – ready channels for distribution of genetically improved lines.

Atlantic salmon

- Sophisticated *nucleus breeding design*:
- Full-sib and paternal half-sib family group produced onshore
- Sea unit → rears breeding candidates
- There's a few sea-unit locations for performance testing
- 4-year generation time → multiple year-classes i evaluation at a time
- High-capital operation!
- Sell broodstock to "*multipliers*"
- Multipliers produce seed-stock for sale to growers, who raise fish for market



(Gjerde et al., nd)

Note that the structure of the genetic improvement program…

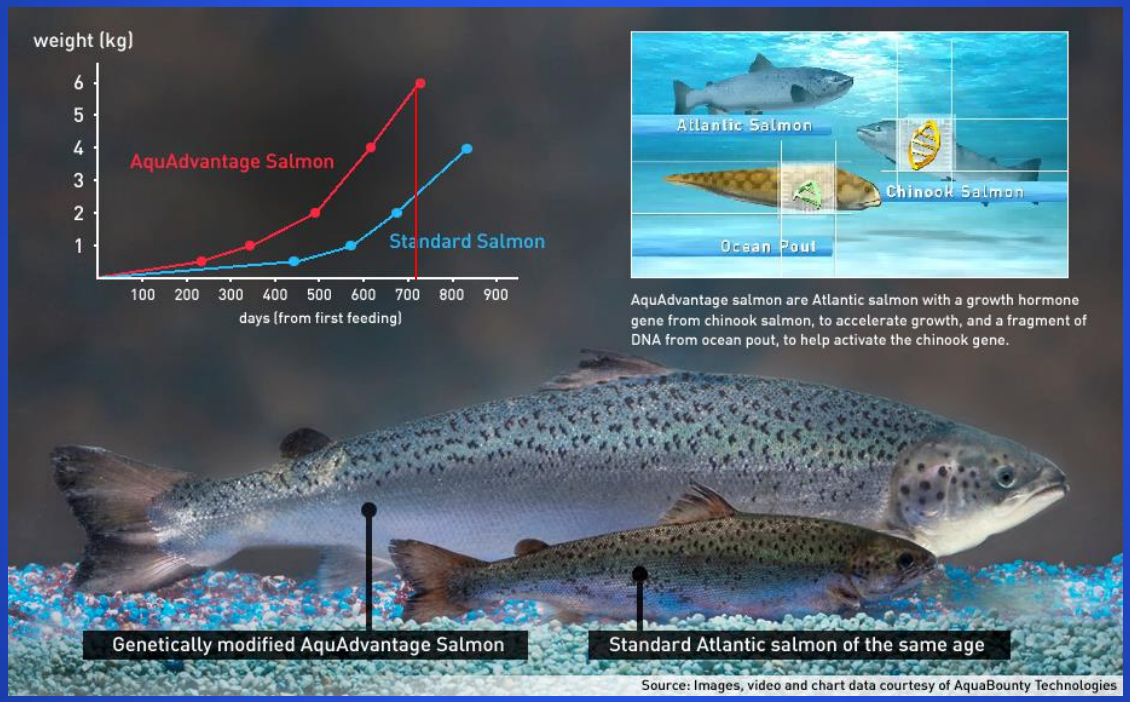
- Nucleus → genetically improved stock
- Multipliers → fingerlings
- Growers → marketable product

Note that the structure of the genetic improvement program… *would be suitable for adoption in West Africa*

- Nucleus → genetically improved stock – university, NGO, or government station
- Multipliers → fingerlings – technically proficient commercial operations
- Growers → marketable product – small producers, including family operations.

What about biotech in the Atlantic salmon sector?

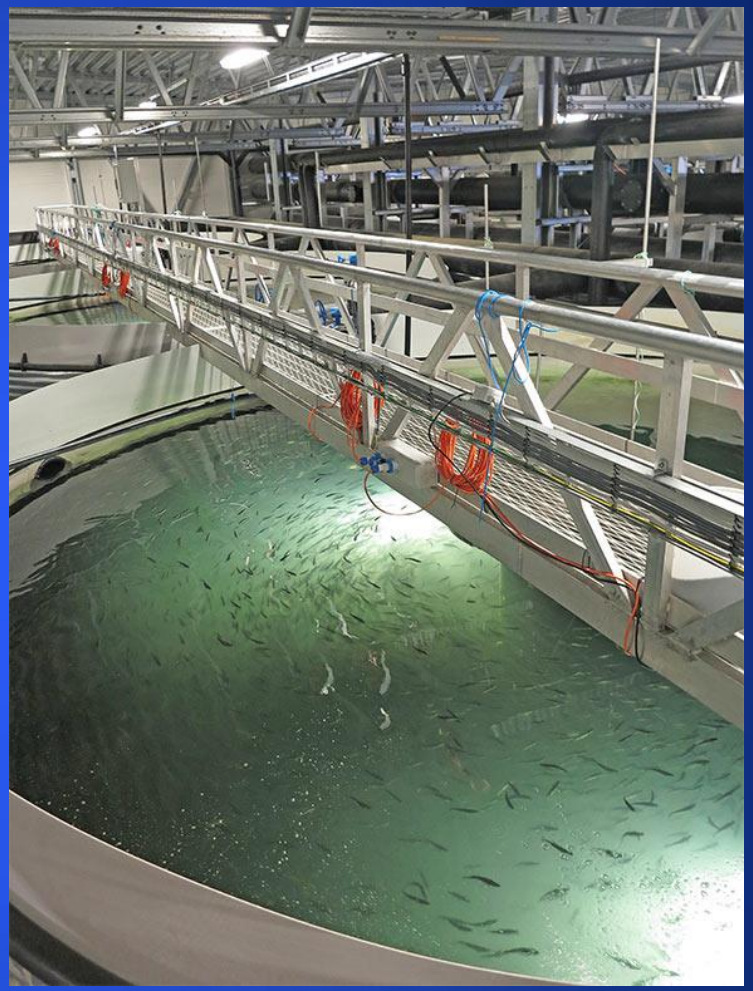
- AquaBounty Atlantic salmon



- After much regulatory delay, *approved* for production in Canada and Panama, for marketing in Canada (USA to follow soon?)

Production in RAS?

- Pre-smolts long have been cultured on-shore
- Can *grow-out* phase of production be shifted onshore?
- Large recirculating aquaculture systems (RAS) are being developed in order to deal with issues regarding sea lice outbreaks (Norway), algae blooms (Chile), and escapes, …
- It is a challenge to break even producing salmon in RAS
- …which may create an opening for producing GM fish under strict confinement in RAS in many countries



Kruger Kaldnes RAS, Veolia Water Technologies

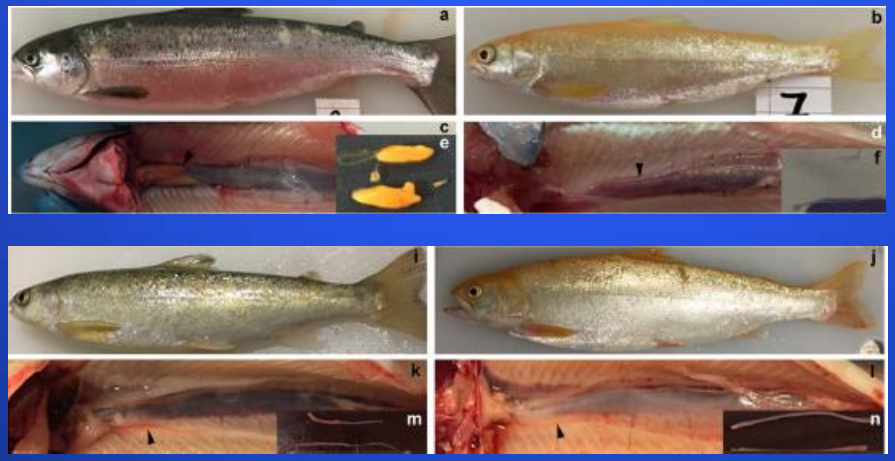
Transgenic reproductive confinement?

- Issue – escape of selectively bred cultured fish into wild, interbreeding with wild fish and loss of local adaptation
- Triploid, female stocks – but 10% reduction of growth rate
- Wargelius et al. (2016) used CRISPR-Cas9 to knock out *dnd*, a gene needed for development of germ cells

Controls *dnd* knockouts

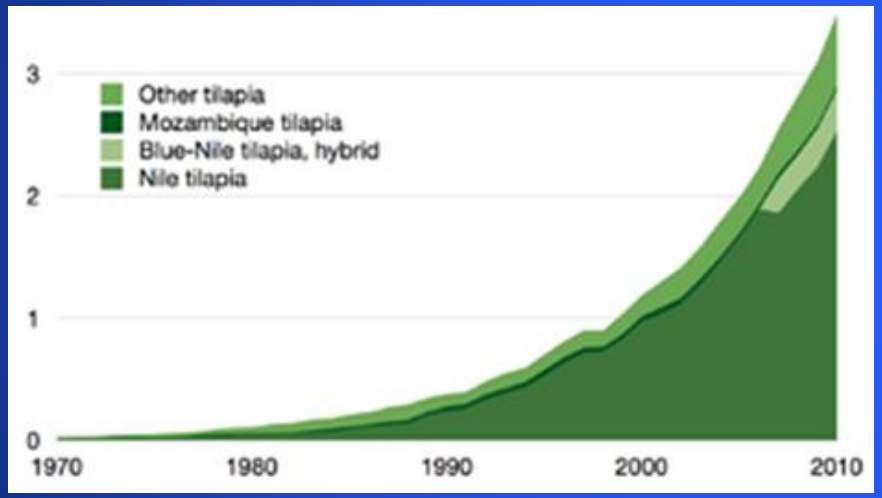
Females

Males



24/30 fish showed knockout and apparent disruption of gonadogenesis

Tilapias



Nile tilapia – *Oreochromis niloticus*

Blue tilapia – *O. aureus*

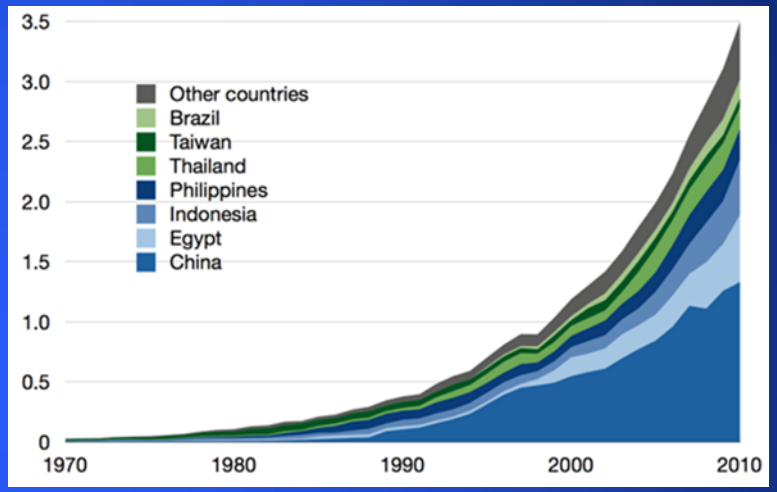
Mozambique tilapia – *O. mossambicus*

Tilapias

- Unique reproductive system; mouth-brooders. Parental care → "anyone" can rear them
- Amenable to production in a variety of systems, from low-input pond aquaculture to super-intensive recirculating aquaculture systems

Tilapias

- Tilapia will feed a billion people and contribute to:
- Food security in Africa and Asia, and
- Commercial production enterprises world-wide



Tilapias

Industry structure:

- Mostly small producers who buy seed-stock from local/regional seed-stock producers.
- *May* or may *not* be genetically improved stock
- May or may not be monosex stocks (males preferred)

- In developed countries, emergence of larger producers with some degree of vertical integration; some have in-house selective breeding programs…

Tilapias

- Because of mouth-breeding habit, easy to spawn and selective breeding is relatively straightforward

E.G.: Selective breeding of fast-growing, white hybrid *O. niloticus* at Blue Ridge Aquaculture, Martinsville, VA, USA

Tilapias

- There are many genetically improved lines…
- Breeders and producers label their strains with the names of institutions (University of Stirling), companies (Blue Ridge Aquaculture), or even individuals (Mike Sipe).

- Lines may be proprietary; may be sold regionally or even internationally,…
- But most have but limited impact.

The GIFT strain

- A notable breeding program, with international impact: the Genetically Improved Farmed Tilapia (GIFT) Project:
- A collaboration between the Philippine Bureau of Fisheries and Aquatic Resources, two Philippine universities, AKVAFORSK of Norway, and ICLARM (now the World Fish Center).
- Germplasm collected from Egypt, Ghana, Senegal, and Kenya, and four Philippine farm stocks → genetically mixed base population → combined family and within-family selection strategy implemented through nine generations (Ponzoni et al., 2011).

The GIFT strain

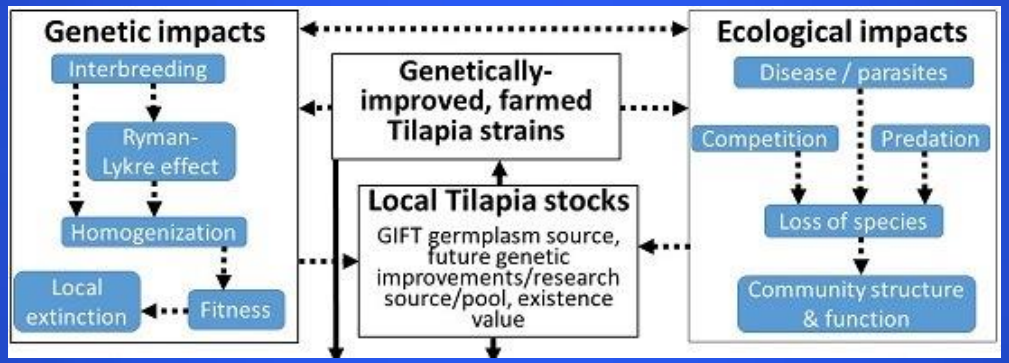
- Genetic gain for growth rate over nine generations was 64% relative to the founding population (Khaw et al., 2008).
- Results of the GIFT project have generated interest from developing countries in Asia, the Pacific, and Africa.
- The GIFT strain first was disseminated to 11 countries in Asia (Gupta and Acosta, 2004).

The GIFT strain

- In 1999, the GIFT Foundation International signed an agreement with GenoMar, a Norwegian company, for long-term continuation of the GIFT breeding program.
- Beginning with the 10th GIFT generation, DNA marker-assisted selection has been applied in order to increase the selection differential by an estimated 40% higher than for 9th-generation GIFT tilapia.
- Genetic maps were generated for tilapia, and experiments were conducted in order to reveal QTLs for color, growth, body shape, salt tolerance, sex determination, disease resistance and feed conversion.
- The “GenoMar Supreme” tilapia are being distributed in countries in Southeast Asia and Latin America.

The GIFT strain

- The WorldFish Center decided *not* to introduce the GIFT strain into countries where O. niloticus is indigenous,



- … but rather to help countries apply the GIFT methodology to genetic improvement of *indigenous* tilapias…

Akosombo strain

- One example is the Akosombo strain, developed in Ghana by the Aquaculture Research and Development Center (ARDEC) in collaboration with the World Fish Center, starting in 2003.
- Progressive pond farmers in Ghana have widely adopted the improved Akosombo strain.
- More work needs to be done to disseminate new strains of tilapia to the majority of pond farmers, who are still farming inferior, mixed, and unknown stocks of tilapia, more than a decade after the development and dissemination of the Akosombo strain started.
- Yaw et al. (2014) showed how small the investment cost of maintaining, continuous improvement, and dissemination of improved strains of tilapia could be relative to the economic benefits of these strains reaching all farmers.

The moment is ripe for new lines

- Work is ongoing to develop regionally derived, selectively bred Nile tilapia lines in Ghana, Nigeria, Kenya, …

Intensifying tilapia production in West Africa

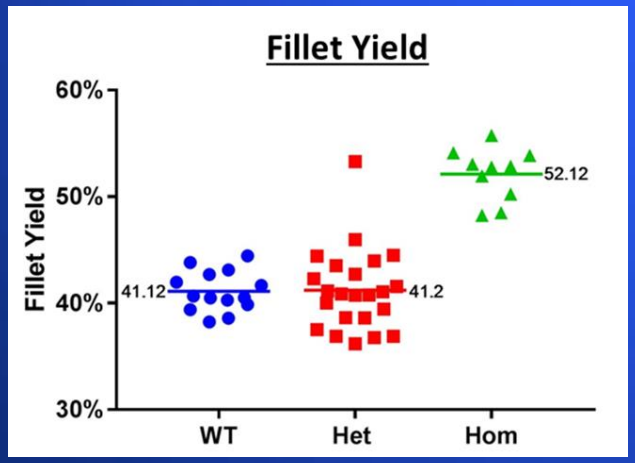
- The AquaFish Innovation Lab (supported by USAID) has observed that for pond farmers growing the Akosombo strain in Ghana:
  - improving pond construction and maintenance,
  - supplementary use of commercial feeds,
  - water quality management,
  - and control of excessive reproduction in ponds (by hormonal sex-reversal and polyculture with a predator)
- …could result in an increase of 2–4 times current average yields using unimproved local strains.

What about biotech in the tilapia sector?

- Growth rate is an important production trait → considerable effort on development of growth hormone-transgenic tilapias:
- Nile tilapia – N. Maclean group (U. Southampton, UK) – *opAFP-sGH* → 2-4x growth rate enhancement
- Hybrid tilapia *O. hornorum urolepis* – M.P. Estrada group (Centro de Ingenieria Genetica y Biotecnologia, Cuba) – hCMV-tiGH → 82% growth rate enhancement
- Current status of these lines?

What about biotech in the tilapia sector?

- Intrexon → tilapia carrying a transgene for fillet yield
  (Peterson, 2017 Animal Biotechnology Regulatory Workshop in Charlottesville)
- The trait is stable across multiple generations
- Tilapia homozygous for the trait demonstrated 53% increase in fillet weight and 27% increase in fillet yield over wild-type fish

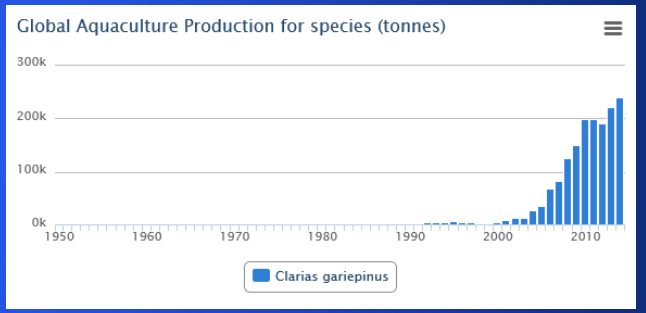


Wild type (left) and homozygous for modified locus (right) siblings

African catfish, *Clarias gariepinus*

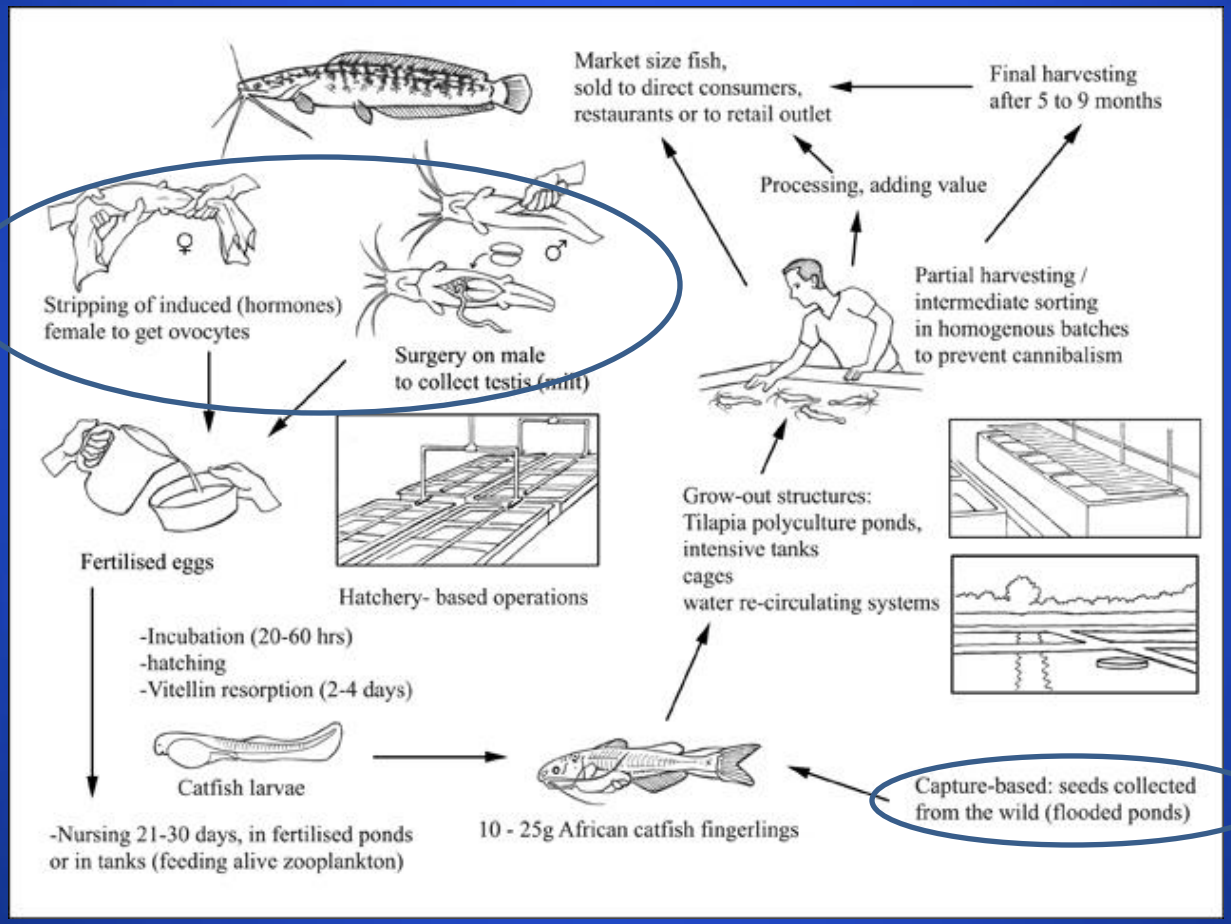
Historical background for culture (FAO 2018)

- Traditional, capture-based culture (known as *wheddos* in Benin and Ghana and *barochois* in Mauritius) goes back centuries
- First modern culture trials – 1950s
- Emergence of commercial culture -1970s
- Hormonal induction of spawning – 1980s
- Subsequent spread of culture regionally and globally
- Regional research programs (Cote d'Ivoire, Nigeria, Central African Republic) → advances including pelleted feeds, tank culture, recirculating aquaculture systems



Production of African catfish

Modern culture



Traditional culture

Production systems

On-growing systems:

- Traditional ponds
- Polyculture in earthen ponds
- Tanks and raceways
- Cages
- Recirculating aquaculture systems

Genetics of African catfish

Very limited work:

- Cryopreservation of spermatozoa (Van der Walt et al. 1993, Viveiros et al. 2000, Omitogun et al. 2012)
- Selection upon a 4 x 4 factorial cross (Volckaert and Hellemans 1999) → no response to selection
- Male-specific DNA markers (Kovacs et al. 2000)
- Gynogenesis (Galbusera et al. 2000)
- Hybridization with *Heterobranchus logifilis* (Ataguba et al. 2009)
- Crossbreeding of farmed and wild *C. gariepinus* (Sunarma et al. 2016)
- No application of gene technology

Much more work needed!

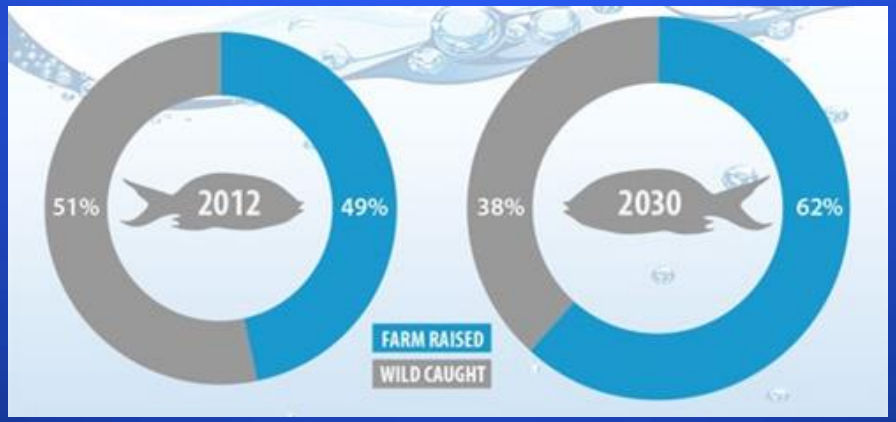
Poor performance of cultured stocks is widely noted

Some key points

- Aquaculture is growing dramatically in importance to human food security in west Africa
- Aquaculture is not one sector, it's a series of independent sectors on very different trajectories
- Use of genetically improved lines:
  - Varies between sectors
  - Depends heavily on culture systems and industry structure
  - Will need to increase to meet need, esp. in Africa

Some key points

- Biotechnology, *broadly construed*, includes hormonal induction of spawning, hormonal sex reversal, and chromosome set manipulation – only hormonal sex reversal of tilapia is widely practiced in west Africa.
- Adoption of gene technology is entirely yet before us.
- Judicious application of biotechnology could contribute to aquacultural productivity, human nutrition, food system resilience, and economic development in west Africa

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Dakar
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The Gambia
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Sierra Leone
Liberia
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