



Adoption of Genetically Improved Farmed Tilapia (GIFT) twenty years after release to industry: *A GIFT that keeps on giving?*

John Benzie and Curtis Lind
WorldFish, MALAYSIA



The GIFT that keeps giving

Genetically Improved Farmed Tilapia (GIFT) has been developed for nearly 30 years to have fast growth, benefiting millions across the world

The project: *Adoption of Genetically Improved Farmed Tilapia (GIFT) twenty years after release to industry: A GIFT that keeps on giving?*

The technology: A selectively bred strain of tilapia developed in the 1990's, known as GIFT, that became highly successful in tropical aquaculture development:

The issue: Effective monitoring of dissemination and adoption poses many challenges. Farmers have no real way to determine genetic origins of their stock.

**What are current estimates of adoption of GIFT and GIFT-derived tilapia strains in Philippines and Bangladesh?
Are they supported by molecular analysis of farm-level tilapia stocks?**



28

Years that GIFT has been developed through selective breeding

20

Number of GIFT generations produced through selective breeding

5

Number of continents farming GIFT

16

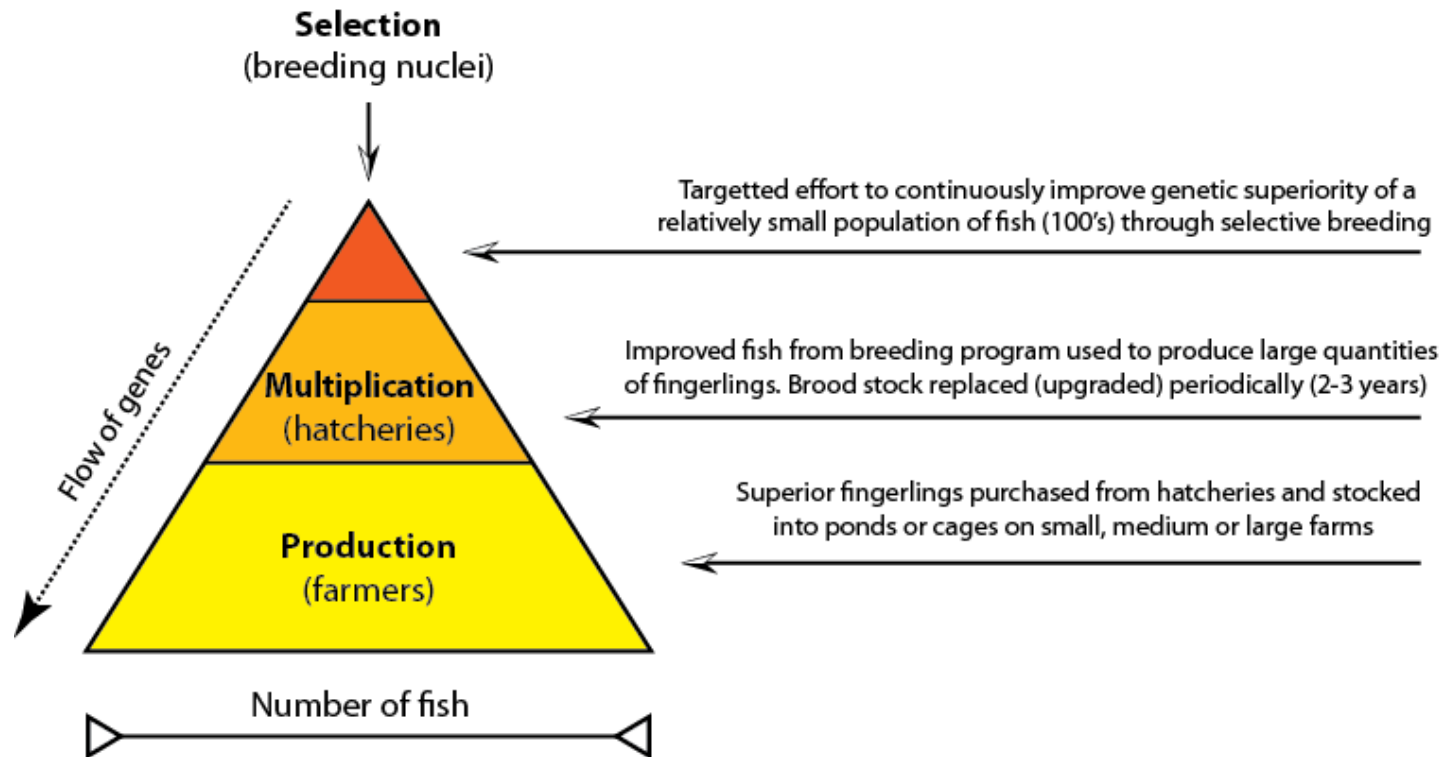
Number of countries WorldFish has distributed GIFT to

The project: *Adoption of Genetically Improved Farmed Tilapia (GIFT) twenty years after release to industry: A GIFT that keeps on giving?*

1. Improve estimates of current extent of adoption through innovative tracking of fingerling diffusion
2. Review and update recent estimations of GIFT and GIFT-derived tilapia adoption rates in Philippines and Bangladesh
3. Characterize nucleus populations of key GIFT and non-GIFT tilapia strains using genome-wide SNP genotyping approaches
4. Validate recently completed field-based estimates of GIFT and GIFT-derived tilapia adoption in Philippines and Bangladesh through SNP genotyping of farm-level tilapia stocks



Dissemination approach for tilapia

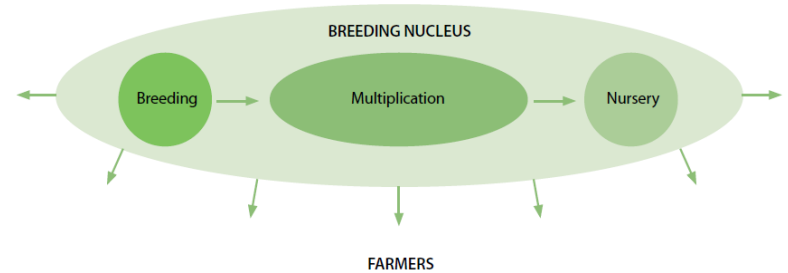
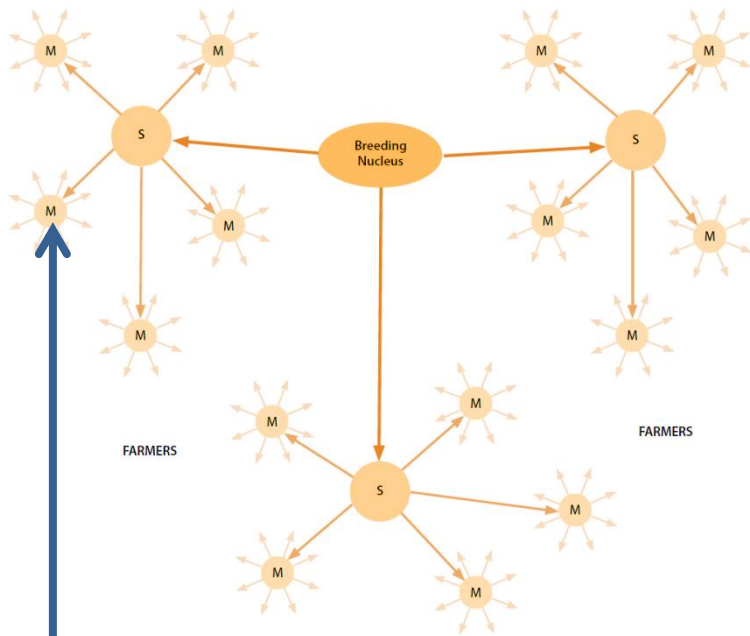


high fecundity and reproductive rate can result in rapid scale-out

tilapia hatcheries may produce >15 million fingerlings per year

ideally functions as a “flush-through” system, farmers do no breeding

Dissemination approach for tilapia



Mixture of centralized (r) and decentralized (l) distribution

Records from breeding nuclei allows tracking and estimation of distribution to multiplication hatcheries

Key factor in being able to estimate dissemination feasibly

Estimating adoption of GIFT

Objective 1: Map geographic patterns of adoption

Objective 2: Review estimates of current adoption

GIS approach piloted in Philippines using public breeding nuclei and multiplication center data

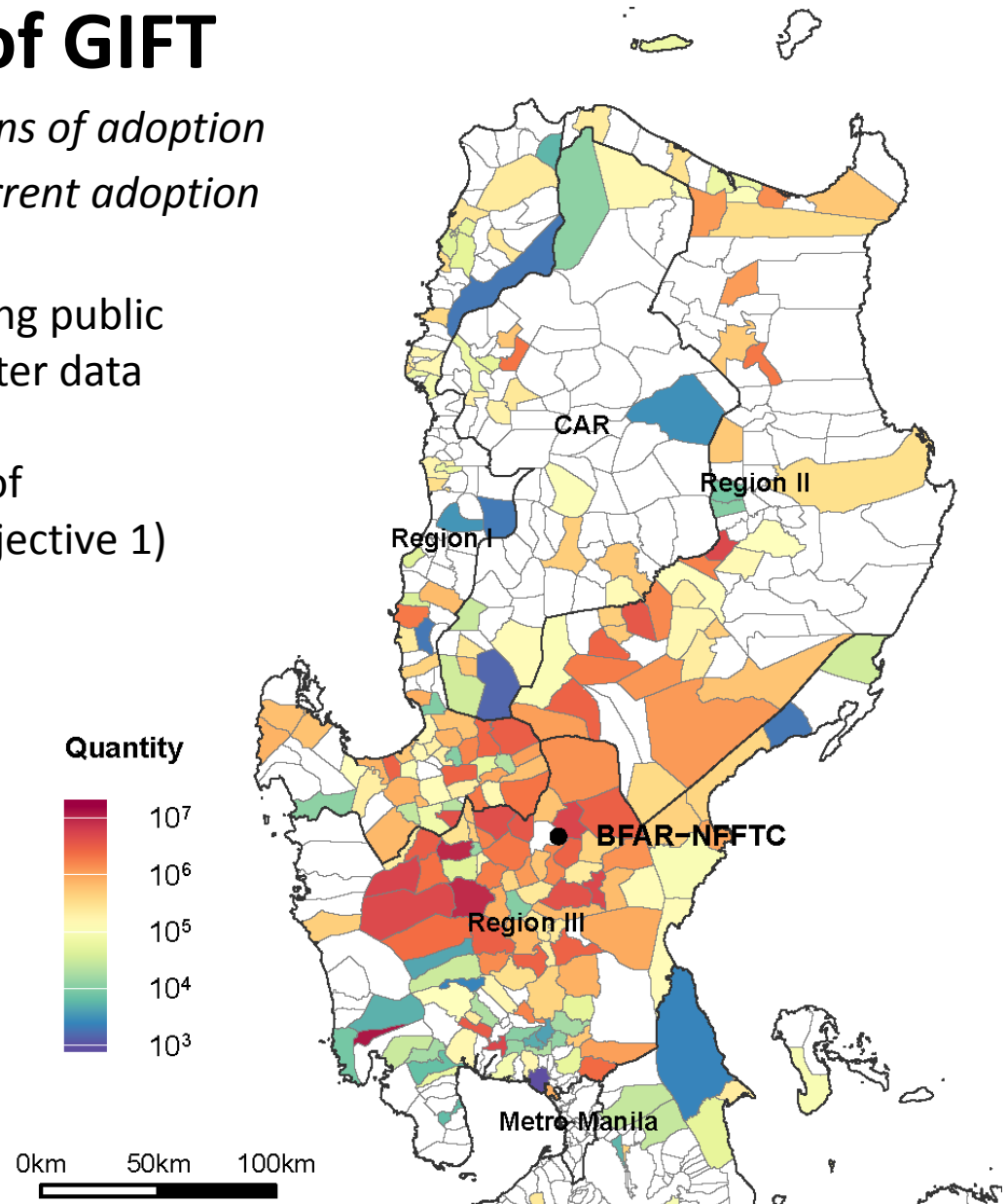
Current project to build on this for all of Philippines and test in Bangladesh (Objective 1)

Complemented by...

- Reviews of recent literature
- Key informant interviews (Objective 2)

To provide:

- Magnitude and extent of GIFT (derived) fingerling production
- Estimates of proportions of different strain adoption
- Estimate of production from *strains of uncertain origin*



Estimating adoption of GIFT

Objective 1: Map geographic patterns of adoption

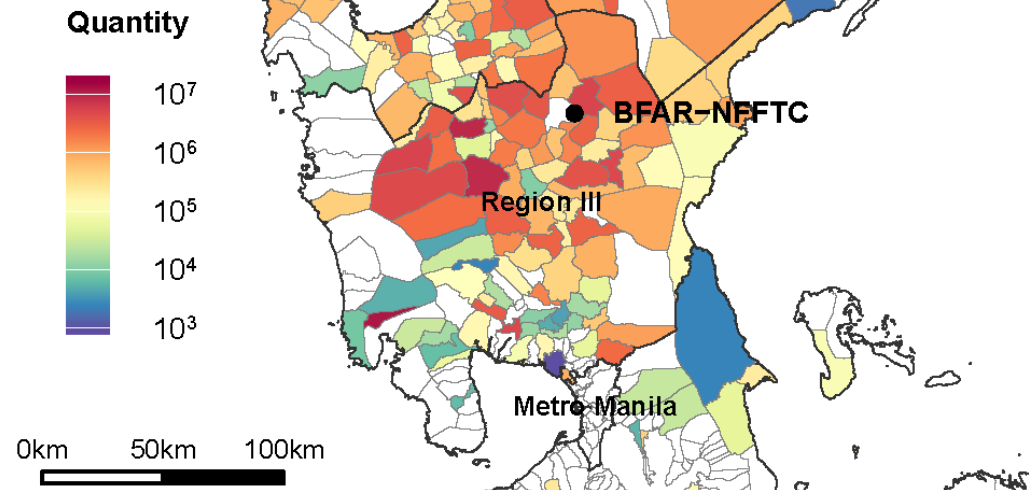
Objective 2: Review estimates of current adoption

Progress to date...

- Reviews of recent literature completed and being compiled
- Detailed dissemination data collected for the main government tilapia distributor in Philippines
- Major field activity in Bangladesh to collect tilapia production and dissemination data from 200 hatcheries (~75% of all hatcheries in country) almost complete

To be done...

- Data cleaning and checking
- Mapping of distribution patterns
- Estimate national tilapia fingerling production in Bangladesh and Philippines
- Key informant interviews



Sampling strategy: molecular component

Objective 3: Characterization of nucleus populations

GIFT nucleus

WorldFish Malaysia, and original
in Philippines



Non-GIFT nuclei

FaST, Chitralada strains



GIFT-derived strain nuclei

GET-ExCEL, BEST, Molobicus strains



STRAIN	SAMPLES	ORIGIN
GIFT-MY	139	GIFT
GIFT-PH	47	GIFT
GET-ExCEL	94	GIFT-derived
BEST	47	GIFT-derived
Molobicus	128	GIFT-derived
FaST	120	Non-GIFT
Chitralada	94	Non-GIFT
TOTAL	669	

21,195 SNPs identified using **DARtseq™** platform and under assessment for use in optimal assignment of hatchery individuals

Nucleus populations of six key strains genotyped with 21k SNP loci

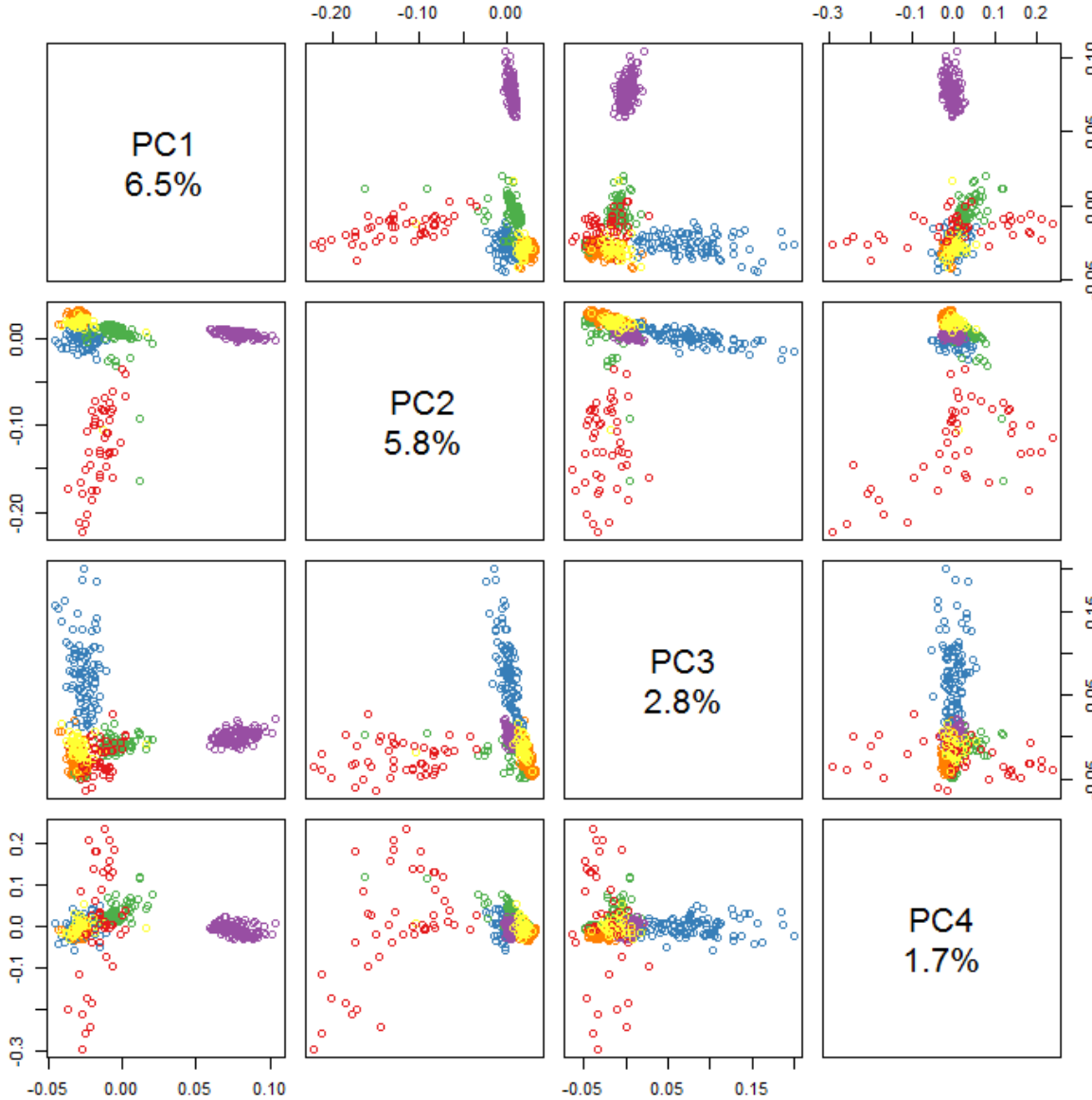
Genotyping approach

- Service provided by **Diversity Arrays Technology** based in Canberra, Australia
- **DArTseq** technology platform used for high resolution genotyping
- DArTseq a genome complexity reduction approach - genomic representations sequenced on Next Generation Sequencing (NGS) platforms
- Cost-effective
- Detailed information on website
- Each individual of the nucleus populations genotyped at **>21,000 SNP loci**, currently being analysed
- A **subset of 1000 SNPs will be selected** for individual assignment of hatchery samples



www.diversityarrays.com

Preliminary results: strain characterization



Indications...

- Distinct separation for some strains
- Others less clear
- Based on simple PCA only with full SNP panel

Continuing with...

- More sophisticated model-based analyses underway
- Targeted SNP panel should improve clustering

	STRAIN	ORIGIN
Orange	GIFT-MY	GIFT
Yellow	GIFT-PH	GIFT
Green	GET-ExCEL	GIFT-derived
Red	BEST	GIFT-derived
Purple	FaST	Non-GIFT
Blue	Chitralada	Non-GIFT

Sampling strategy: hatchery component

Objective 4: Genetic origins of hatchery-level tilapia stocks

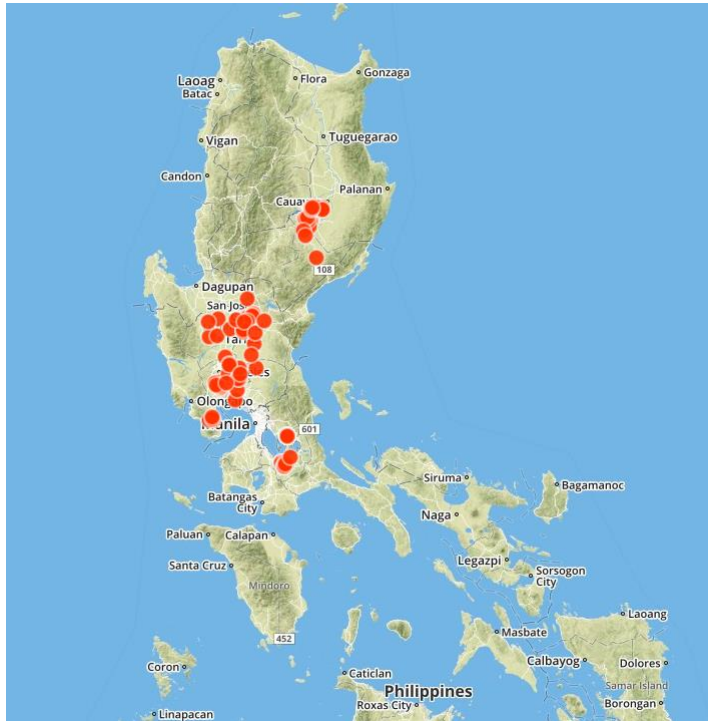
	PHILIPPINES			BANGLADESH			Total
Main producing regions	Central and Northern Luzon			Dhaka, Khulna and Chittagong Divisions			
Believed genetic origin	GIFT or GIFT derived	Non-GIFT	Uncertain	GIFT or GIFT derived	Non-GIFT	Uncertain	
No. hatcheries	41	23	45	61	8	44	222
Fry/Fingerlings produced in 2015 (millions)	210	86	258	355	85	308	1302
Tissue samples per hatchery	10-20	10-20	10-20	10	10	10	
Total samples	560	330	600	610	80	440	2620

- Each individual to be genotyped at 1000 highly informative SNPs
- Individual-based clustering and genetic assignment
 - STRUCTURE (Pritchard et al. 2000)
 - Discriminant Analysis of Principal Components (DAPC, Jombart et al. 2011)
 - NETVIEW (Network analysis based on pairwise similarities)
- ***Sampling complete, genotyping to commence shortly***

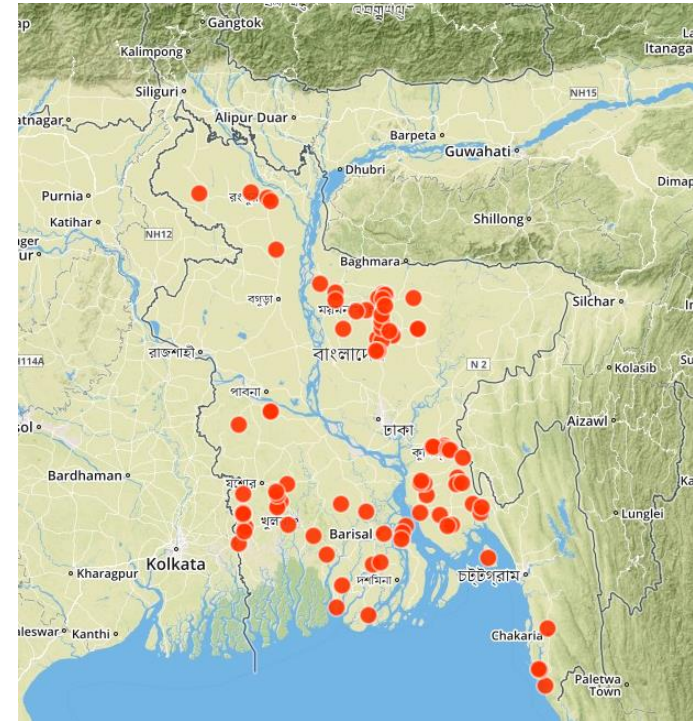
Progress to date: Hatchery sampling

Objective 4: Genetic origins of hatchery-level tilapia stocks

PHILIPPINES



BANGLADESH

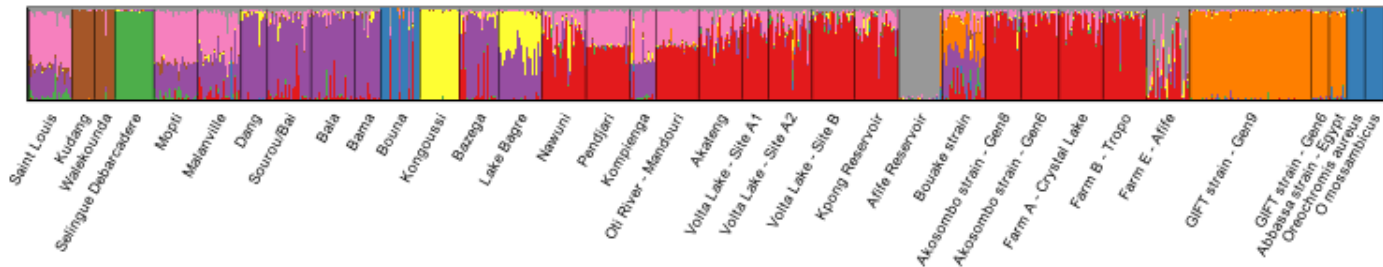


2015 Production	Hatcheries sampled	Fingerlings (millions)
Uncertain	45	258.4
Non-GIFT Derived	23	85.6
GIFT Derived	41	210.1
Total	109	554.1

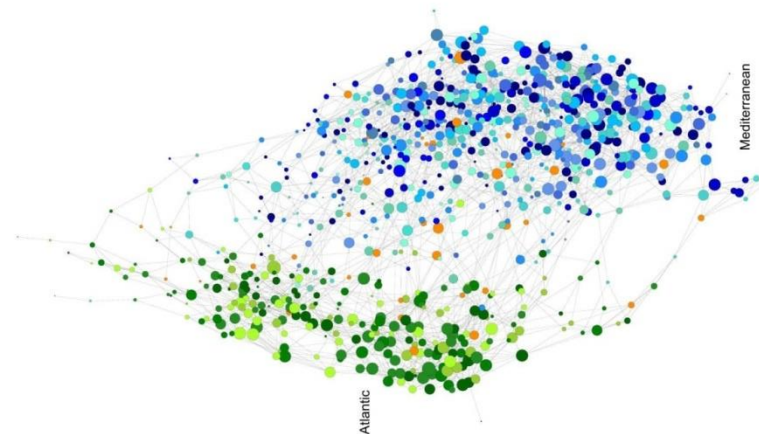
2015 Production	Hatcheries sampled	Fry (millions)
Uncertain	44	308.3
Non-GIFT Derived	8	84.5
GIFT Derived	61	354.7
Total	113	747.5

How will genotype information be used?

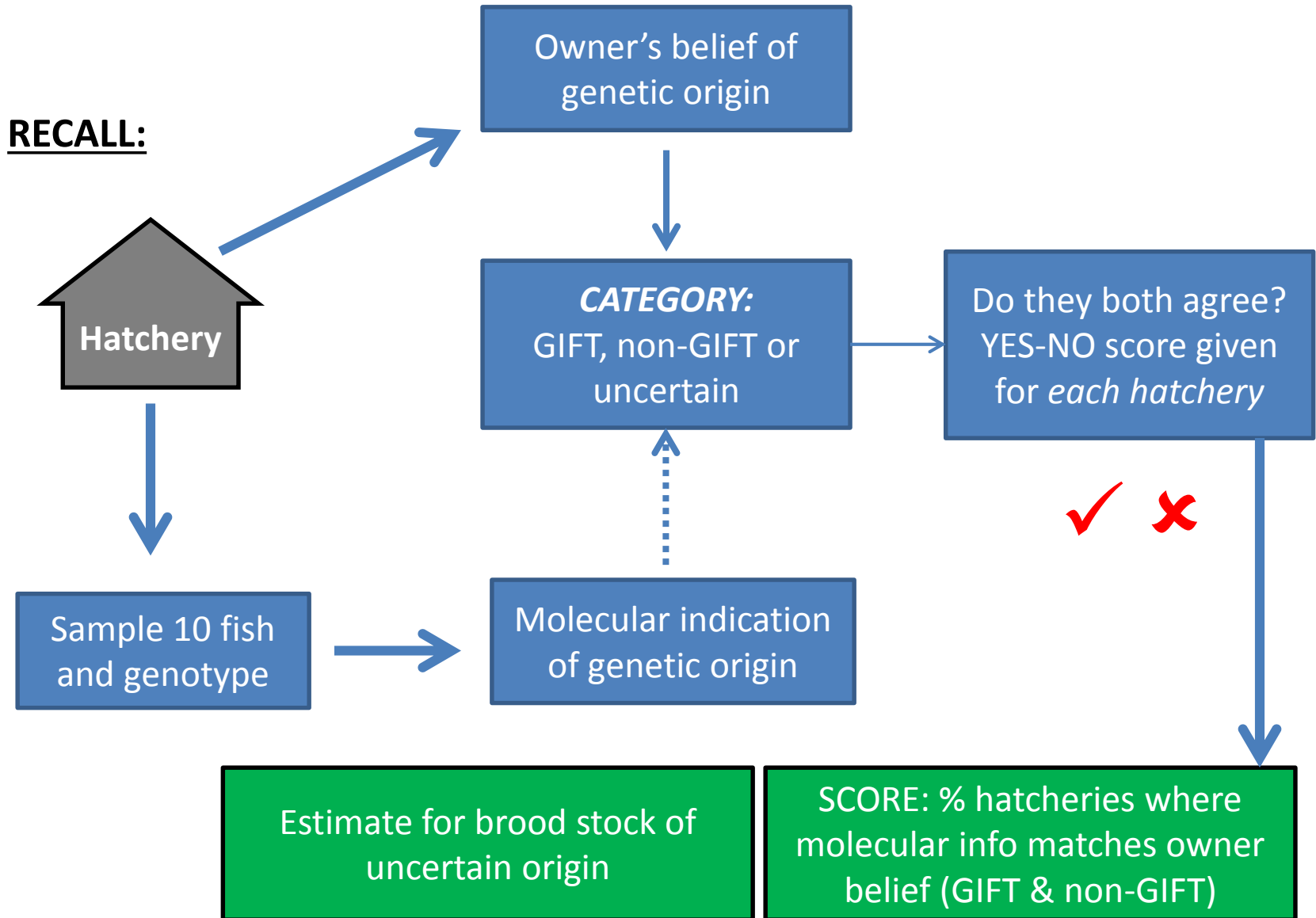
- Each hatchery broodstock sampled genotyped for 1000 highly informative SNPs selected from a broader pool of 21,195 SNPs (DartSeq platform)
- Individual-based clustering and genetic group assignment
 - STRUCTURE (Pritchard et al. 2000)
 - Discriminant Analysis of Principal Components (DAPC, Jombart et al. 2010)



- NetView P - Network analysis based on pairwise similarities (Steinig et al. Mol. Ec. Res in press)



How will genotype information be used?



How will genotype information be used?



TRADITIONAL ESTIMATE OF ADOPTION

Literature review
KI interviews
Government statistics



factor to adjust traditional
estimates

Estimate for brood stock of
uncertain origin

SCORE: % hatcheries where
molecular info matches owner
belief (GIFT & non-GIFT)

REVISED ESTIMATE
OF ADOPTION



Challenges encountered

LOGISTICAL:

Production and dissemination data available but inconsistently and sporadically recorded by national aquaculture centers

ANALYTICAL:

Preliminary analyses of genotype data indicates some strains many not clearly distinguishable from each other

PRACTICAL:

Cleaning large quantities of dissemination data is highly time consuming (transcribing from scanned written notebooks of hundreds of hatcheries)

Slow responses for delivery of promised data in a number of cases

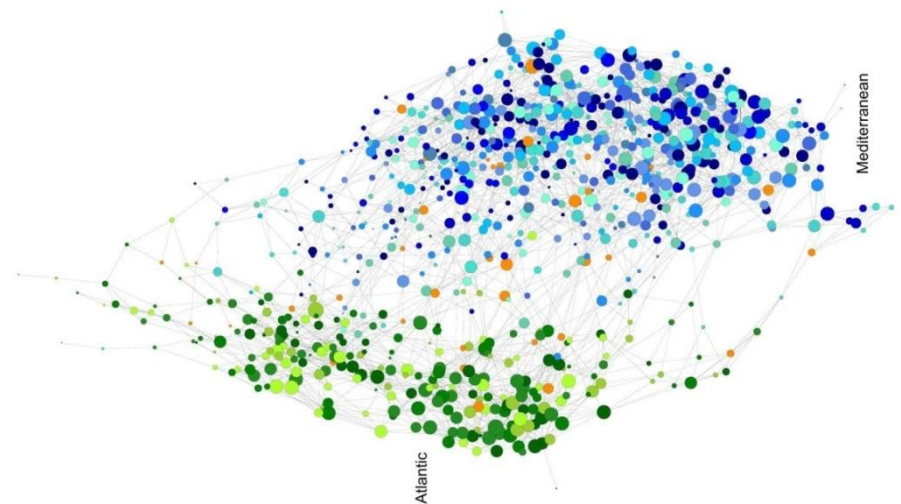
Staff movements have disrupted workflow and timelines

Points for discussion

If strains are not distinguishable using >20k SNPs, should they be considered as different even if development histories are well known?

Criteria for cut-off points for assigning hatchery-sampled individuals to one strain or another may be confounded by the above point

Estimating national fingerling production is a challenge. Reasonable estimates are likely to be derived from average hatchery size and number of fingerlings per breeder, but remains a source of uncertainty



Thank you



Contact:

Curtis Lind

c.lind@cgiar.org

John Benzie

j.benzie@cgiar.org



Independent
Science and
Partnership
Council

Sampling strategy: data collection and storage

WorldFish Sample Collection

Welcome!

WELCOME TO THE WORLD FISH SAMPLE COLLECTION DATA ENTRY FORM:
(version 7, last updated 20-May-2019)

Basic information

NAME OF COLLECTOR:

PROJECT NAME:

RECORD YOUR LOCATION:
(Click on the "target" icon to initiate GPS. Strongly recommended to do this just before beginning inventory)

latitude (x,y ")
38.902522

longitude (x,y ")
-77.051917

altitude (m)

accuracy (m)

Sample information

DATE OF COLLECTION:
yyyy-mm-dd

SPECIES NAME
(select one)

Roho (Labeo rohita)

Mola (Amblypharyngodon microlepis)

Hilsa (Tenualosa ilisha)

Nile tilapia (Oreochromis niloticus)

Mrigal (Cirrhinus cirrhosus)

Other

TYPE OF TISSUE COLLECTED:
(select one)

Finclip

Muscle tissue

- Mobile data collection tools (**OpenDataKit, KoboToolbox**) to gather essential project data
- Browser based (multi-platform) with offline capability
- Barcoding system for tissue collection tubes
- Pre-printed labels provided to collectors
- Ethanol storage for archive in WorldFish biorepository (LIMS)



Sampling strategy: molecular component

Objective 4: Genetic origins of hatchery-level tilapia stocks

	PHILIPPINES			BANGLADESH			Total
Main producing regions	Central and Northern Luzon			Dhaka, Khulna and Chittagong Divisions			
Believed genetic origin	GIFT or GIFT derived	Non-GIFT	Uncertain	GIFT or GIFT derived	Non-GIFT	Uncertain	
No. hatcheries	35	35	35	35	35	35	210
Tissue samples per hatchery	10	10	10	10	10	10	
Total samples	350	350	350	350	350	350	2100

- Each individual genotyped for 10k SNPs (DartSeq platform)
- Individual-based clustering and genetic assignment
 - STRUCTURE (Pritchard et al. 2000)
 - Discriminant Analysis of Principal Components (DAPC, Jombart et al. 2011)
 - NETVIEW (Network analysis based on pairwise similarities)