Advancing Aquaculture Systems Productivity Through Carp Genetic Improvement

Fish Innovation Lab

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Partners/Institutions

Academic partners

- Bangladesh Agricultural University
- Louisiana State University

WorldFish G3 Rohu dissemination partners (hatcheries)

- Bangladesh Department of Fisheries (DoF)
- Bangladesh Fisheries Research Institute (BFRI)
- Afil Aqua Fish Ltd
- Aftab Bahumukhi Farms Ltd
- Bhai Bhai Matsya Hatchery
- Building Resources Across Communities (BRAC)
- Fishtech Hatchery Limited
- Jamuna Fish limited
- Ma Fatima Fish Hatchery
- Matri Fish Hatchery
- Mukteshary Fish Hatchery
- Rupaly Fish Hatchery
- Sonar Bangla Hatchery & Farms Ltd
- Tushar Matshya Hatchery

WorldFish G3 Rohu on-farm performance trial partners (grow-out farmers)

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- Ali-Abdullah Dairy Farm
- Ashroy Trainning Center
- Fahad Hatchery and Fish Farm
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- Molla Fish Nursery and Dairy Farm
- Muttakim Traders
- Osit Matsya Khamar
- Razib Kumar Sarkar
- Sagor Fish Hatchery
- Saifujjaman Pintu

Abbreviations and Acronyms

AGGRC Aquatic Germplasm and Genetic Resources Center

BAU Bangladesh Agricultural University

BFRI Bangladesh Fisheries Research Institute

BRAC Building Resources Across Communities

CGIP Carp Genetic Improvement Program

DoF Bangladesh Department of Fisheries

G0 Generation zero (i.e. base / unimproved population)

G1 Generation one (first selected generation)

G2 Generation two (second selected generation)

G3 Generation three (third selected generation)

LSUAC Louisiana State University Agricultural Center

SNP Single nucleotide polymorphism

Glossary

All definitions are from Gjedrem and Baranski (2009)

Aquaculture Production of aquatic animals or plants under farming conditions.

Artificial selection Selection of parents to reproduce for the next generation based on particular criteria by which we decide which animals shall be the parents that reproduce to make the next generation.

Base population The individuals giving rise to a population (e.g. the individuals used for starting a breeding program).

Breeding goal A list of traits which shall be improved in a breeding program and their relative importance.

Breeding station The facility or unit where a breeding program is carried out.

Breeding value The additive genetic performance of an individual according to the breeding goal.

Broodstock Animals that are selected to be parents for the next generation.

Combined selection The simultaneous use of records from an individual itself, its relatives and their pedigree relationships for selection.

Control A standard used for comparison or an experiment established as a check of other experiments.

Dam A female parent.

Dissemination of genetic gain The transfer of selectively bred (genetically improved) animals from the breeding station to the industry and the use of those animals, or their progeny, by the industry for production purposes.

Egg A female gamete.

Full-sibs Individuals that have both a common father and a common mother.

Gamete A haploid sex cell, sperm or egg.

Gene The unit of heredity, or a segment of the DNA molecule containing information that can be transcribed and translated into proteins.

Generation interval The average age in years of parents when their progeny are born.

Genetic gain The change in the value of a trait due to selection (caused by the change in allele frequencies in response to selection).

Genotype-by-environment interaction (G×E) When the relative genetic performance of individuals or genetic groups (e.g. sib families or populations) for a given trait changes from one test environment to another.

Inbreeding The mating of animals sharing common ancestry (related animals).

Inbreeding depression The reduction in performance caused by inbreeding.

Monoculture The separate culture of different species.

Multiplier A farm cooperating with the breeding station to multiply and disseminate the improved stock to the industry.

Pedigree A diagram or matrix showing the genetic relationship between family members.

Pedigree-based selection The selection of animals based on the performance of their parents, grandparents and/or other relatives.

PIT tag Passive Integrated Transponder tag, an electronic tag for identification purposes, usually inserted into the body cavity.

Polyculture The mixed culture of different species in the same farm unit.

Population A separate group of animals within a species.

Relationship The proportion of the genetic material shared by two individuals as a result of inheritance, or the classification of individuals according to their common ancestry (e.g. sibs, progeny).

Sample A restricted number of individuals chosen from a larger group according to a rule (often at random).

Selection The choice of parent broodstock from a larger group of candidates based on their breeding value, to produce a new generation with improved performance.

Sire A male parent.

SNP Single Nucleotide Polymorphism, genetic variation in a DNA sequence that occurs when a single nucleotide in a genome is altered.

Spawning When aquatic species release mature eggs or sperm from the gonads.

Species A group of animals that are freely able to breed with one another but are unable to freely breed with other species in the wild.

Sperm A male gamete.

Strain A population of individuals with a common genetic history and often some common characteristics, sometimes called a breed in farmed species.

Trait A character or state that may be recorded in an individual (e.g. growth rate, flesh tenderness, feed conversion ratio, disease resistance).

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Abstract

WorldFish manages pedigree-based genetic improvement programs for three significant aquaculture species – catla (*Labeo catla*; syn. *Catla catla*), rohu (*Labeo rohita*) and silver carp (*Hypophthalmichthys molitrix*) – in Bangladesh. Using these genetically improved populations, the 'Advancing Aquaculture Systems Productivity Through Carp Genetic Improvement' project aimed to assess the on-farm performance of a generation three (G3) rohu multiplier population (Output 1); disseminate additional genetically improved carp (Output 2); produce new generations of improved carp (Output 3); develop a cost-effective pedigree assignment tool (Output 4), produce practical manuals, tools, and undertake training activities (Output 5); and create a cryopreserved sperm repository strategy for commercial carp genetic improvement and dissemination (Output 6).

Under the project, genetically improved generation three (G3) rohu, generation one (G1) catla, and generation two (G2) silver carp families were produced (Output 3) – it is anticipated that each new generation will grow approximately 10% more rapidly than the previous generation. Furthermore, these populations were backed up at two geographically distant backup sites maintaining representative live fish (Output 3) and possible approaches for future cryopreservation of sperm were articulated in a sperm repository strategy (Output 6).

Realized genetic gains, for the breeding goal of rapid growth, in the G3 rohu multiplier were quantified (harvest weight for G3 rohu was ~37% greater than the unimproved control) through trials conducted on 'real-world' grow-out farms. Results of these trials indicated substantial improvements in pond productivity would be possible through the widespread dissemination and adoption of genetically improved carp (Output 1). To this end, the Project disseminated the G3 rohu multiplier to additional hatcheries. At the conclusion of the project the 59 hatcheries known to maintain G3 rohu broodstock.

The dissemination of genetically improved G3 rohu through commercial hatcheries was monitored. In 2022, commercial hatcheries sold 245 kg of G3 rohu spawn (i.e. hatchlings) to nurseries and farmers at a substantial premium – approximately three times the price of their standard rohu products. By the conclusion of the project these fish were being grown on approximately 3082 farms (Output 2). Sales of G3 rohu spawn by commercial hatcheries increased substantially in 2023, to 2826 kg, and are anticipated to increase further in 2024 and beyond, as more brood fish become sexually mature and increase in size.

The Project has also provided G3 rohu performance data and training to hatchery owners to improve their understanding of genetics, and strengthen marketing and business decision making in the sector (Output 5).

A 118 Single Nucleotide Polymorphism (SNP) panel for pedigree assignment in rohu was developed (Output 4). However, when validation of this SNP panel was attempted, it was concluded that a panel comprised of more SNP, and/or SNP with a greater average minor allele frequency (MAF), would be required for routine parentage assignment. Based on the experience in rohu, a catla panel, of 486 SNP (of known minor allele frequency in the WorldFish population) was developed, but is yet to be validated.

The research undertaken, training provided, and technologies produced and disseminated under this project, have improved the capacity of hatcheries to manage and market genetically improved carps; and will improve pond productivity, farmers' incomes and the supply of fish, as genetically improved carp are increasingly adopted. Furthermore, the backup populations established, and the cryopreservation strategy developed, under this project will improve the security of genetically improved carp populations; and the further development, refinement and implementation of the pedigree assignment tools will simplify management and enhance genetic gains achieved in carp genetic improvement programs.

Introduction

WorldFish manages pedigree-based genetic improvement programs for three significant aquaculture species – catla (*Labeo catla*), rohu (*Labeo rohita*) and silver carp (*Hypophthalmichthys molitrix*) – in Bangladesh. The WorldFish Carp Genetic Improvement Program (CGIP) commenced with the collection of catla and rohu founders, as spawn, from Bangladeshi rivers in 2012 (Hamilton et al., 2019a; Hamilton et al., 2019b) (Figure 1). Subsequently, silver carp founders were collected, as adults, from 17 Bangladeshi hatcheries in 2014 (Hamilton et al., 2021). Base populations were then generated by mating between founders (2014 for rohu, 2016-2017 for catla and 2017 for silver carp). The first generation spawned from selected parents (i.e. first artificially-selected generation) was produced in 2014 for rohu (Hamilton et al., 2022a), 2021 for catla and 2019 for silver carp (Hamilton et al., 2023b). All WorldFish CGIP activities are undertaken at a hatchery, grow out and broodstock rearing facility (i.e. breeding station) near Jashore, Bangladesh.

Spawning seasons within the 'Advancing Aquaculture Systems Productivity Through Carp Genetic Improvement project

	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
Rohu	F		Base		G1		G2		**G3**	G3	
Catla	F					Base				G1	G1
Silver carp			F			Base		G1			G2

Rohu G3 multiplier released to hatcheries as hatchlings

Figure 1. History of the WorldFish Carp Genetic Improvement Program – 'F' indicates the year in which founders were collected; 'Base' is the spawning season in which unselected founders were mated to produce base-population (G0) families; and 'G1', 'G2' and 'G3' represent spawning seasons in which generation one, generation two and generation three families, respectively, were produced from parents selected for rapid growth (i.e. harvest-age weight). Note that rohu and silver carp have a generation interval of two years and catla has a generation interval of three years.

Using the WorldFish genetically improved carp populations, the ‘Advancing Aquaculture Systems Productivity Through Carp Genetic Improvement’ project (herein referred to as ‘The Project’) aimed to produce the following outputs:

- Output 1: Performance analysis of genetically improved rohu disseminated to hatcheries and farmers
- Output 2: Dissemination of additional genetically improved carp species to partner hatcheries to be developed into broodstock
- Output 3: New generations of improved carps
- Output 4: A cost-effective pedigree assignment tool
- Output 5: Practical manuals, tools, and training activities
- Output 6: A cryopreserved sperm repository strategy for commercial carp genetic improvement and dissemination in Bangladesh

Research Methods

Output 1: Performance analysis (on-farm trials)

Prior to the commencement of The Project, fry from the 2020 G3 rohu multiplier population (Figure 1) were nursed at the WorldFish CGIP Facility. The G3 rohu multiplier population was comprised of high-ranking G3 rohu families that were not closely related.

Under The Project, in May 2021, these fish were distributed to 19 semi-commercial farms across two Bangladeshi 'regions' (Jashore and Natore–Rajshahi), to compare the on-farm performance of the G3-multiplier against fish from the WorldFish control line – genetically equivalent to fish sourced from rivers – and a well-regarded commercial strain. Fish were then grown out under 'real world' conditions prior to harvest in April-May 2022, at which point they were assessed for weight. Using these data, the realised genetic response to selection (i.e. realised genetic gain; Gjedrem and Baranski, 2009) for harvest weight in the G3 Rohu multiplier was estimated (Hamilton and Yeasin, 2021; Hamilton et al., 2022b; Yeasin et al., 2022).

Output 3: New generations of improved carps

The breeding cycle adopted in the WorldFish CGIP is summarised in Figure 2. The completion of one breeding cycle represents one generation of selection.

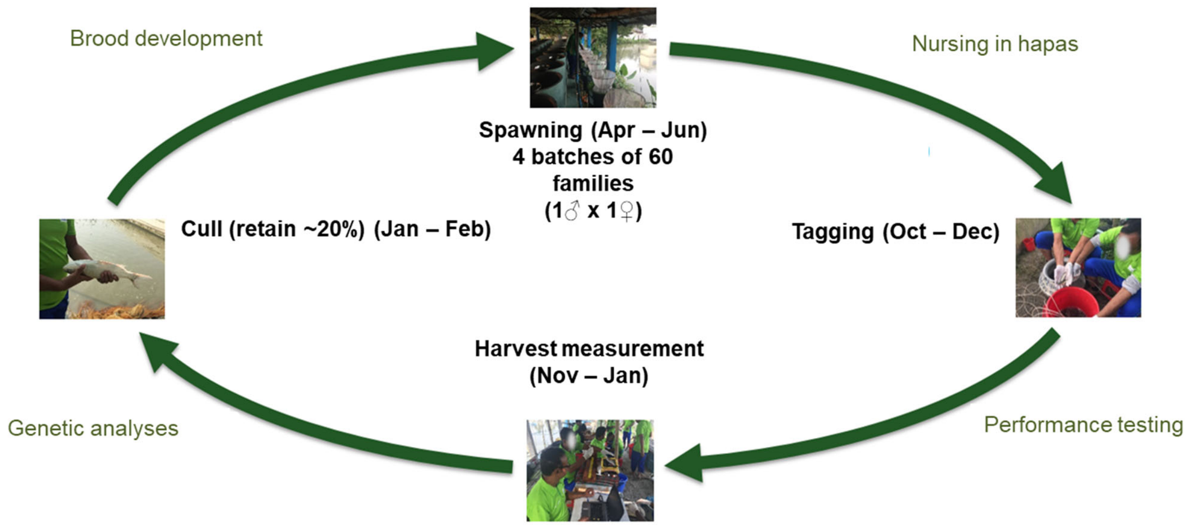

Figure 2. WorldFish carp genetic improvement program breeding cycle

Under The Project, the WorldFish Carp Genetic Improvement Team (Appendix 1), produced full-sibling families for each of three lines – 'selection', 'control' and 'negative' – of G1 catla, G3 rohu, and G2 silver carp across two spawning seasons (Figure 1). Parents were selected using combined selection on the basis of estimated breeding values (EBVs) for harvest-age weight and readiness to spawn at the time of spawning (Hamilton et al., 2022a), while constraining average relatedness (an indicator of future inbreeding; Meuwissen, 1997) within each line. Parents of selection lines were selected for the breeding goal of rapid growth (i.e. individuals with high EBVs for harvest-age weight were selected), parents of the control lines were selected to have an average EBV for weight of zero (i.e. to maintain a population genetically equivalent to the unimproved base populations) (Hamzah et al., 2014), and parents of the negative lines were selected for slow growth.

Fry from each full-sibling family spawned in 2021 and 2022 were nursed in hapas (i.e. nets; one family per hapa and one hapa per family). Once fish were of a size large enough for tagging, a passive integrated transponder was inserted into 40 randomly-selected fingerlings, and the 10 largest remaining fingerlings, in each family with sufficient fish (Table 1). Tagged individuals were then transferred to grow out ponds for performance testing in full-sibling family trials at the WorldFish Carp Genetic Improvement Facility (Figure 2). Full-sibling family trials are a form of common-garden experiment (Ljungfeldt et al., 2014) and involve the comparison of different full-sibling families under identical environmental conditions (i.e. within a pond). Feeding and species composition across ponds was varied to allow examination of genotype-by-environment interaction (Table 2) (Hamilton et al., 2023b).

Table 1. WorldFish Carp Genetic Improvement Program full-sibling families made in 2021 and 2022 spawning seasons.

Year spawned	Line	Catla (G1) families	Rohu (G3) families	Silver carp (G2) families
2021	Control	17	35	0
	Negative	15	15	0
	Selection	90	178	0
2022	Control	10	0	32
	Negative	0	0	16
	Selection	71	0	192

Table 2. Number of fish by year, pond and species included in full-sibling family trials established or maintained under The Project at the WorldFish Carp Genetic Improvement Facility. Some ponds were managed under monoculture and others under biculture (i.e. polyculture involving two species).

Year spawned	Pond	Pond Area (m^2)	Supplementary pelletized feed	Catla individuals	Rohu individuals	Silver carp individuals
2020	TAL_09	5099	True	0	2918	0
2021	TAL_11	2266	True	2007	0	0
	TAL_13	2266	True	0	2046	0
	TAL_16	1335	True	0	1206	0
	TAL_17	1780	True	1593	0	0
	TAL_23	1254	True	0	1134	0
	TAL_26	2711	True	1083	1344	0
	TAL_27	2590	True	0	2340	0
	TAL_31	2792	True	1156	1389	0
2022	TAL_07	2064	False	0	0	1765
	TAL_08	1295	True	718	0	549
	TAL_14	1902	False	0	0	1624
	TAL_15	2347	False	1302	0	1001
	TAL_18	1942	False	0	0	1623
	TAL_19	1335	False	742	0	572
	TAL_20	1376	False	0	0	1178
	TAL_21	2468	True	1371	0	1055

At harvest age, all surviving fish spawned in 2020 and 2021 were assessed for total weight, standard length to silvering, width and depth (Holden and Raitt, 1974). Data for standard length, width and depth were recorded primarily to allow changes in the shape of fish to be monitored over the long term (i.e. across generations). Genetic analysis were undertaken to generate estimated breeding values for harvest age weight, according to the methods detailed in Hamilton et al. (2022a). These EBVs were then used to identify fish to be retained for development into brood (i.e. to identify candidate parents for the next generation) (Figure 2).

Output 4: A cost-effective pedigree assignment tool

<u>Rohu</u>

To develop and validate a cost-effective pedigree assignment tool:

- the DArTseqLD platform was adopted for genotyping. DArTseqLD was developed by Diversity Arrays Technology (DArT) and is a *de novo* genotyping-by-sequencing (GBS) method capable of generating high-quality low-density SNP data (Kilian et al., 2012), and
- a 'pooling-for-individual-parentage-assignment' method, aimed at reducing the cost of pedigree assignment (Hamilton, 2021), was applied and tested.

Fin-clip samples from a total of 720 individuals from 48 G3 rohu families (15 individuals per family) spawned in 2020 were genotyped along with their G2 parents (96 parents), as were 264 additional parents of non-genotyped G3 families. Tissue samples from G3 individuals (i.e. the progeny) were allocated to wells on 96-well plates for genotyping – 720 wells containing a single tissue sample from one individual; 360 wells containing tissue samples from two individuals; and 240 wells containing tissue samples from three individuals. Tissues samples from G2 individuals (i.e. possible parents) were allocated to 360 wells containing a single tissue sample from one individual (Hamilton M. G. et al., in prep).

<u>Catla</u>

A targeted genotyping by sequencing method (DArTag) was adopted for catla. Although this approach has a higher up-front cost, it provides more certainty of obtaining a SNP panel that is fit for purpose than non-targeted GBS approaches, such as DArTseqLD. A panel of 486 previously-identified SNP were targeted for inclusion on the panel. In addition 14 putative "sex markers" – catla sequences that map to sex-associated fragments in rohu identified in a previous Fish Innovation Lab project (Arick et al., 2023) (Quick Start Grant ID 7200AA18CA00030) – were also included in the panel.

A total of 2068 samples were genotyped using this panel. These data will be used to determine if:

- parentage of catla individuals can accurately and unambiguously be assigned using SNP markers (maximum likelihood method of assignment applied to separate early rearing (SER) fish) (Hamilton, 2021),
- parentage of catla pools of two individuals can accurately and unambiguously be assigned using SNP markers (maximum likelihood method of assignment applied to SER fish) (Hamilton, 2021),
- there are equal numbers of individuals per family at tagging (within spawn runs) under communal early rearing (CER) (Ninh et al., 2011),
- the genetic correlation between CER harvest weight and SER harvest weight equals one (likelihood ratio test) (Gilmour et al., 2014), and

- if sex can be determined, prior to sexual maturity using the 14 putative sex markers (Arick et al., 2023).

Research Results

Output 1: Performance analysis (on-farm trials of the G3 rohu multiplier)

Trial results were published as a peer-reviewed paper (Hamilton et al., 2022b). Briefly, for harvest weight, i) no significant strain-by-region – a type of genotype-by-environment – effect was evident; ii) the G3-multipier was the most rapidly growing strain across all farms, and iii) realised genetic gains of 38.6% and 34.9% were observed for the G3 rohu multiplier over the control strain in the Jashore and Natore–Rajshahi regions respectively.

Output 3: New generations of improved carps

Results from full-sibling family trials of G3 rohu spawned in 2020 and 2021 (Figures 3a and 3b) were in keeping with i) those observed in other finfish species (reviewed by Gjedrem and Rye, 2018); ii) expectations at the commencement of the WorldFish CGIP – a 10% improvement over the base population was anticipated with each generation of selection (Keus et al., 2017) – and iii) on-farm performance trials of a G3 rohu multiplier population (Hamilton et al., 2022b). Results from full-sibling family trials of G1 catla spawned in 2021 (Figure 4a) were also consistent with genetic gains of 10% per generation for growth rate, but full-sibling family trials of G1 silver carp gains were smaller (Figure 4b). However, the standard errors of the estimated means in G1 trials were large (Figures 4a and 4b) and estimates of genetic gain based on them should be interpreted with caution.

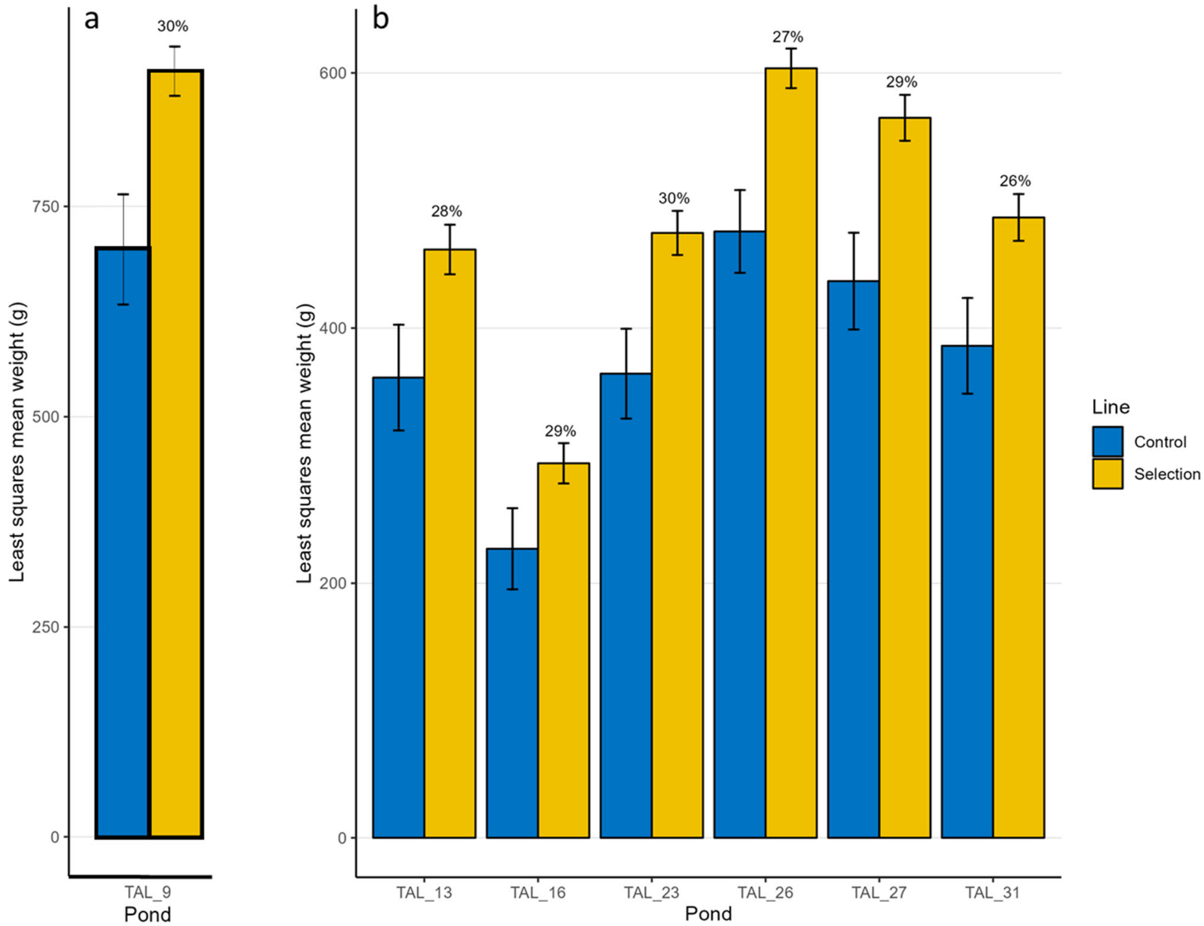

Figure 3. Harvest-age weight by grow out pond for generation three (G3) rohu spawned in 2020 (a) and 2021 (b) and tested in full-sibling family trials at the WorldFish Carp Genetic Improvement Facility. The selection line mean, expressed as a percentage of the control line mean, is indicated for each pond.

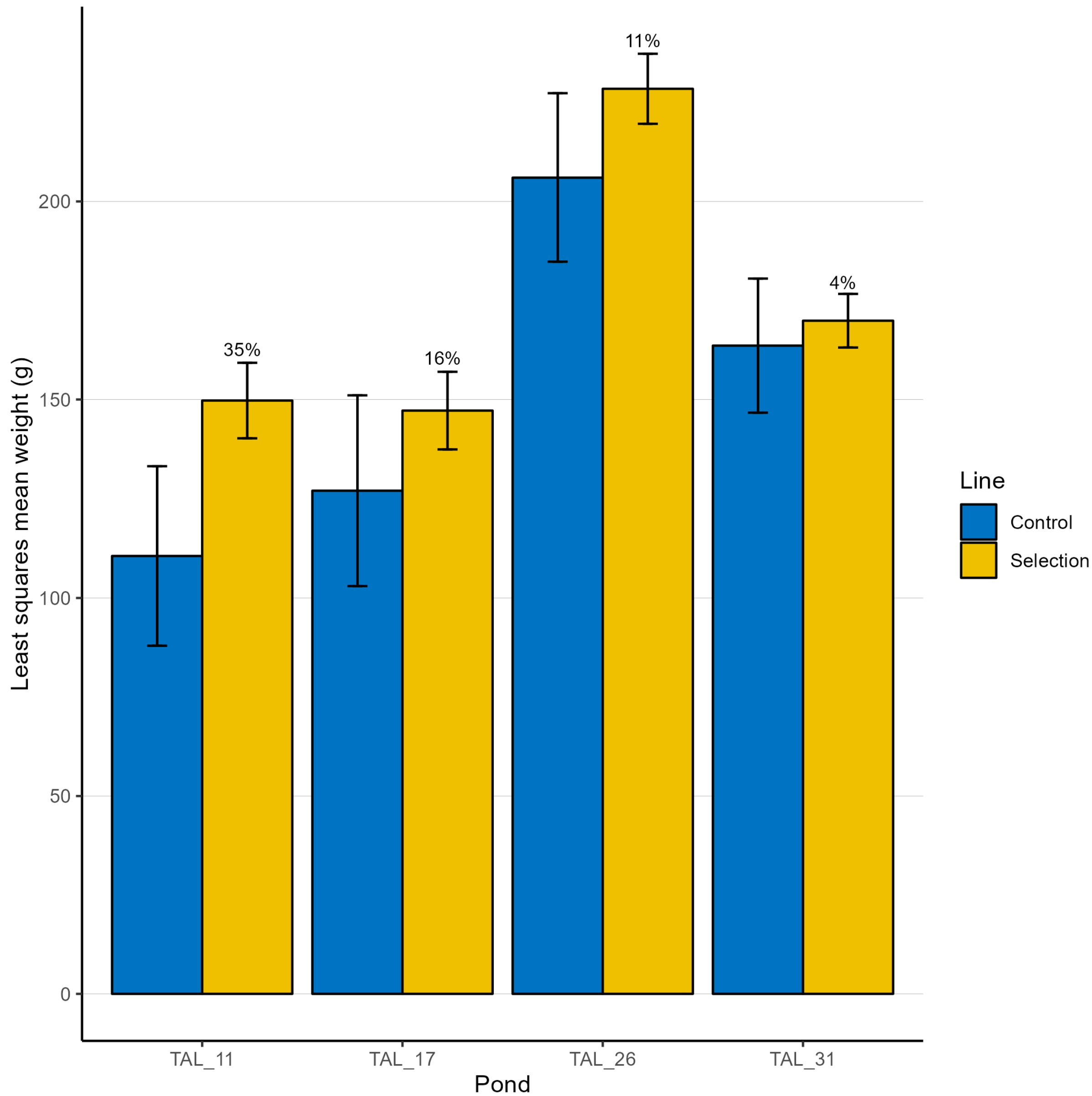

Figure 4a. Harvest-age weight by grow-out pond for generation one (G1) catla spawned in 2021 and full-sibling tested in trials at the WorldFish Carp Genetic Improvement Facility. The selection line mean, expressed as a percentage of the control line mean, is indicated for each pond.

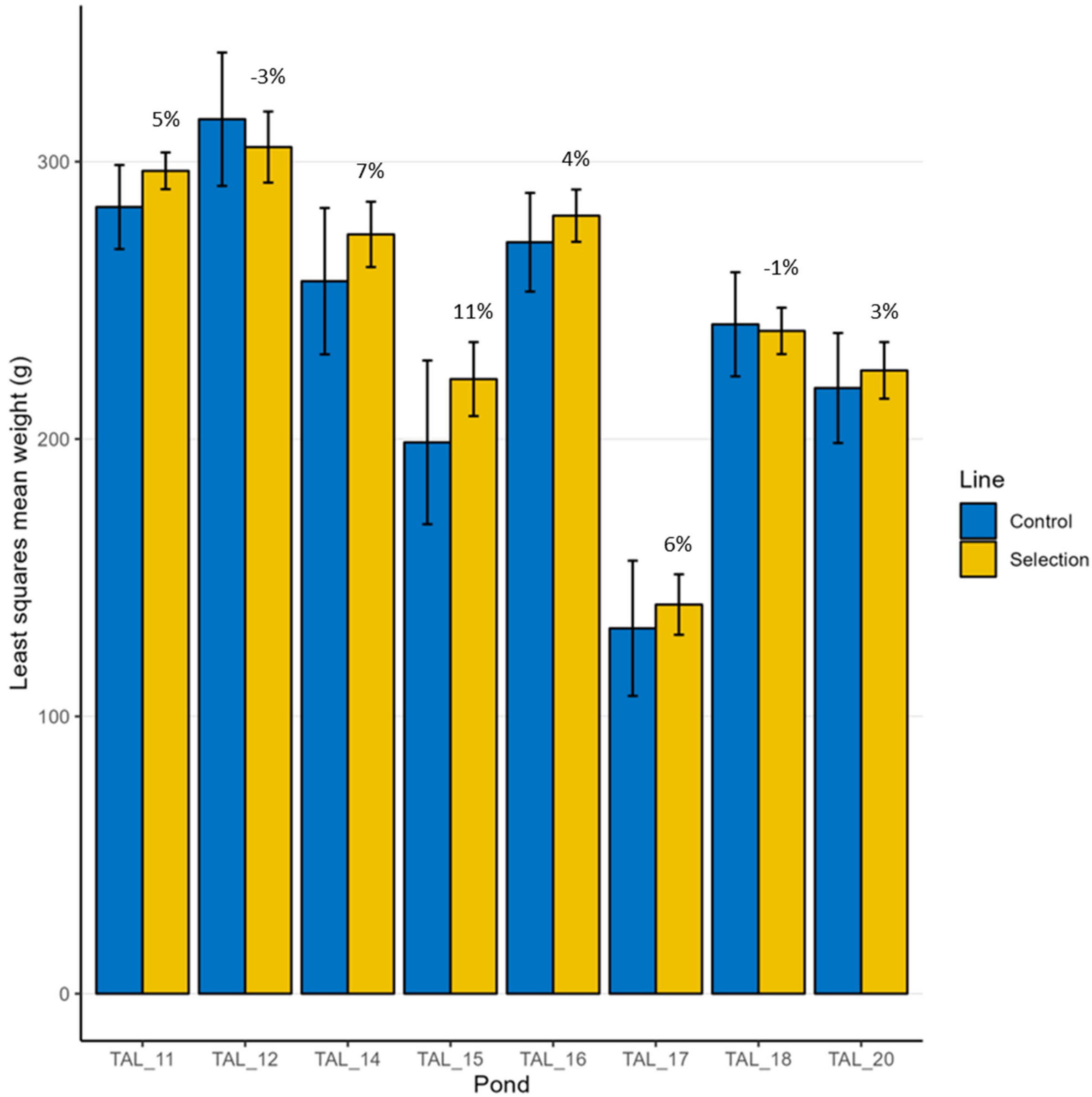

Figure 4b. Harvest-age weight by grow-out pond for generation one (G1) silver carp spawned in 2019 and full-sibling tested in trials at the WorldFish Carp Genetic Improvement Facility. The selection line mean, expressed as a percentage of the control line mean, is indicated for each pond.

Output 4: A cost-effective pedigree assignment tool

Rohu

A smaller than anticipated number of SNPs (i.e. 638) were identified using DArTseqLD and only 118 were retained after quality control, despite the imposition of a relaxed minor allele frequency (MAF) threshold (i.e. ≥ 0.05). Using the Δ LOD values determined by simulation to achieve a correct assignment rate of 95%, for samples comprised of individual progeny, 453 (63%) of individual assignments were accepted of which 77% were determined to be correctly assigned based on pedigree. However, it is possible that these results reflected pedigree or sample tracking errors, rather than assignment errors. It was concluded, a panel comprised of more SNP, and/or SNP with a greater average MAF, is required for routine parentage assignment and the investigation of putative pedigree errors in the population. A paper based on this work will be submitted to a peer reviewed journal (Hamilton M. G. et al., in prep).

Catla

Genotyping data is yet to be analysed but will result in one or more papers and the utilization of animals reared under common early rearing (CER) as parents in the 2024 family spawning of catla.

Outputs and Conclusions

The Project genetically improved core populations of carp with the production of G3 rohu, G1 catla, and G2 silver carp families (Table 1). Furthermore, it secured these populations, with the establishment of two geographically distant backup sites (Figure 5), and provided the foundation for future cryopreservation of sperm for backup with the development of a cryopreserved sperm repository strategy for WorldFish genetically improved carp (Bodenstein et al., 2023). Preparation of the strategy drew on Bangladeshi and international expertise and methods developed through the

Fish Innovation Lab ‘Cryogenic Sperm Banking of Indian Major Carps and Exotic Carps for Commercial Seed Production and Brood Banking’ project.

Realised genetic gains for growth rate in the G3 rohu multiplier (~37% for G3 rohu over the unimproved control) were quantified from trials conducted on real-world grow out farms (Hamilton et al., 2022b) indicating substantial improvements in pond productivity would be possible through the widespread dissemination of genetically improved carp. To this end, the Project monitored the dissemination of genetically improved G3 rohu through commercial hatcheries (Hamilton et al., 2023a). In 2022, The Project has also provided data (Hamilton et al., 2022b) and training to hatchery owners (Hamilton et al., 2022d) to improve their understanding of genetics, and strengthen marketing and business decision making in the sector.

In 2022 and 2023, commercial hatcheries sold 245 kg and 2826 kg, respectively, of G3 rohu spawn (i.e. hatchlings) to nurseries and farmers at a substantial premium – approximately three times the price of their standard rohu products (Hamilton et al., 2023a). Commercial harvest of fish spawned in 2022 will commence in 2023, at which point their enhance growth rate will contribute to farmers’ incomes and the supply of rohu in the market.

In rohu, a 118 single nucleotide polymorphisms (SNPs) panel for pedigree assignment of individual progeny was identified. However, accurate parentage assignment using this panel was not possible due to the high proportion of SNPs of low minor allele frequency (Hamilton M. G. et al., in prep). It is anticipated that a larger catla panel, of 486 SNP (of known minor allele frequency in the WorldFish population), developed under the project, will allow routine parentage assignment in the WorldFish catla genetic improvement program.

As a result of the research undertaken and technologies produced under this project, Bangladeshi hatcheries and nurseries will have improved capacity for management of carp genetics and improved carps. Furthermore, it is anticipated that grow out pond productivity will be improved, and farmers’ incomes will increase with the wide-scale adoption of genetically improved rohu in coming years. In the longer term, the development and refinement of the pedigree assignment tools and cryopreservation strategy developed under this project will also improve the genetic management and security of genetically improved carp populations.

Technologies/Innovations developed, and what phase was achieved

A cost-effective pedigree assignment tool (Phase 1)

Output 4: A cost-effective pedigree assignment tool

The Project identified a 118 SNP panel for rohu using DArTseqLD and developed a 500-marker DArTag panel (486 SNP and 14 putative sex markers) for catla.

Cryopreserved sperm repository (Phase 1)

A cryopreserved sperm repository strategy for WorldFish genetically-improved carp was developed by Louisiana State University Agricultural Center (LSUAC), Bangladesh Agricultural University (BAU) and WorldFish (Output 6). This was published as a publicly-accessible technical report (Bodenstein et al., 2023).

Commercial production of genetically improved carp (Phase 4 for rohu only)

Phase 1: New generations of improved carps

Under The Project, full-sibling families for each of three lines – 'selection', 'control' and 'negative' – of G1 catla, G3 rohu, and G1 silver carp were made across two spawning seasons – 2021 and 2022 (Tables 1 and 2). Selection line families represent the genetically improved population – positively selected for greater harvest weight (i.e. growth rate). Control line families are maintained as an unimproved (i.e. genetically equivalent to the base population) reference point to assist in quantifying genetic gains in performance trials (refer to Output 1). Negative line families will be utilized in future research to create 'F2 mapping populations' to identify quantitative trait loci and genes associated with growth rate in these species (Baranski et al., 2010). New generations of improved carp were secured by sending and maintaining representative individuals to two backup sites (i.e. living gene banks; Figure 5) in the north and northwest of Bangladesh (Table 4).

Table 4. Tagged individuals sent to external backup sites in the north (N) and northwest (NW) of Bangladesh by spawning year, line and species

Year spawned	Line	Catla (G1)		Rohu (G3)		Silver carp (G1 & G2)	
		NW	N	NW	N	NW	N
2019	Control					101	
	Negative					13	
	Selection					459	
2020	Selection				141		
2021	Control	21	54	82	129		
	Negative	4	44	33	65		
	Selection	159	343	381	602		
2022	Control	14	21			17	11
	Negative					8	6
	Selection	66	93			110	87

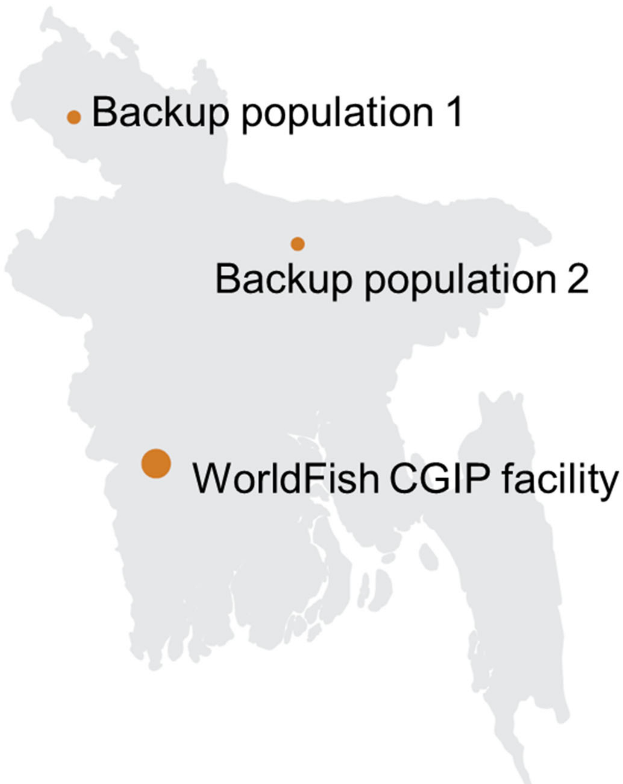

Figure 5. Location of WorldFish Carp Genetic Improvement Program backup sites

Phase 2: Performance analysis (on-farm trials of the G3 rohu multiplier) (Output 1)

On farm performance trials of the G3 rohu multiplier, supplied to commercial hatcheries in 2020, were completed (Hamilton et al., 2022b) and results disseminated (see below).

Phase 3: Dissemination of G3 rohu multiplier populations to hatcheries (Output 2)

To disseminate genetic gain, in May 2021, Worldfish supplied four 'partner' hatcheries with 2.5 kg of G3 rohu multiplier spawn to be grown into broodstock (Figure 6). This was in addition to the seven partner hatcheries supplied with 2.2 kg of G3 rohu multiplier spawn in 2020 (i.e. prior to the commencement of The Project). Agreements with partner hatcheries obliged them to provide WorldFish with details of sales of broodstock to additional 'non-partner' hatcheries (Figure 7a).

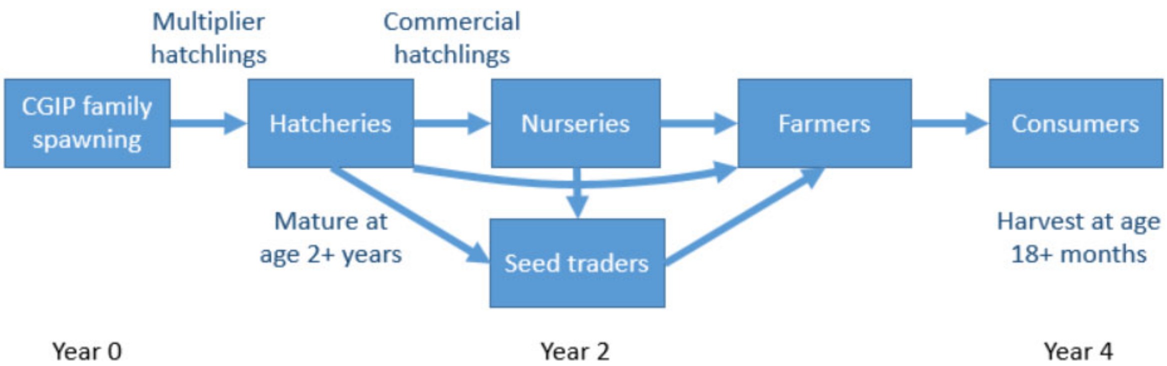

Figure 6. A schematic of the G3 rohu dissemination pipeline.

In June-August 2023, 24 private hatcheries (11 of which did not previously maintain G3 rohu), 12 Department of Fisheries hatcheries, one Bangladesh Fisheries Research Institute (BFRI) hatchery and one educational institution hatchery were supplied with G3 multiplier fish spawned in 2023. Furthermore, the BFRI hatchery was provided with 52 mature G3 multiplier fish from the 2020 spawning. At the conclusion of The Project 38 geographically-disparate private, 18 DoF, one BFRI and two educational institution hatcheries were known to maintain G3 rohu broodstock (Figure 7b).

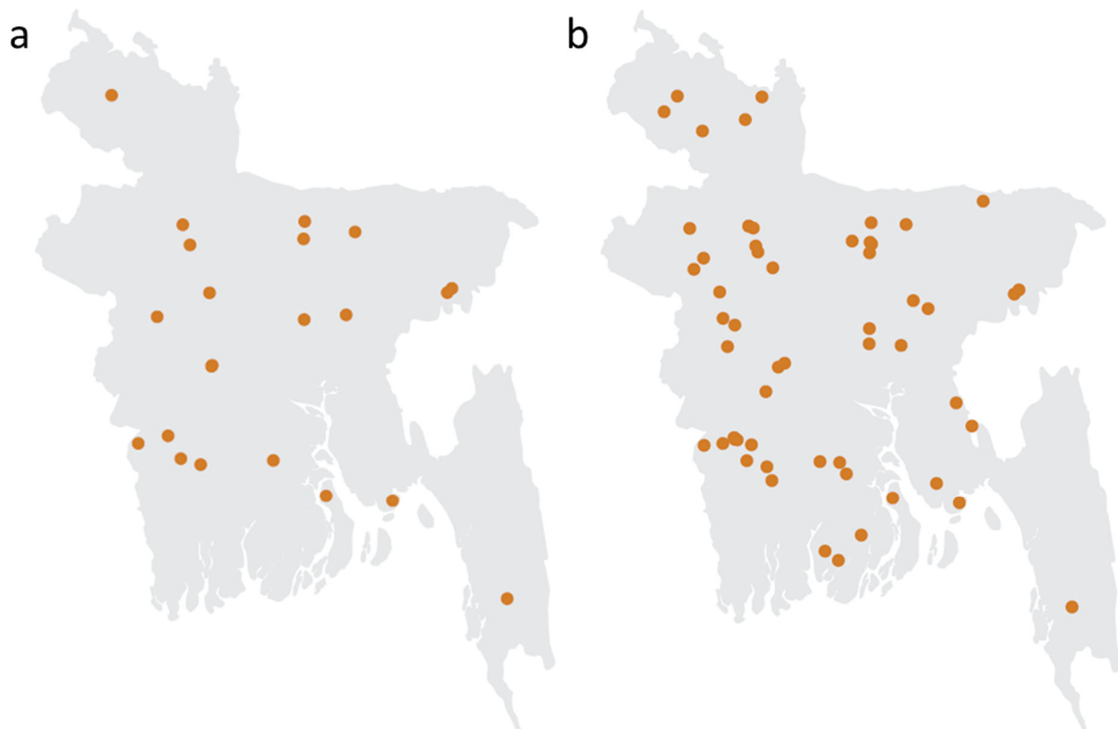

Figure 7. Hatcheries known to maintain G3 rohu multiplier broodstock in a) April 2023 and b) August 2023. Note that some points overlap due to the close proximity of some hatcheries to each other.

Phase 4 Commercial spawning of the G3 rohu multiplier (Output 2)

Commercial Bangladeshi hatcheries sold genetically improved rohu seed for the first time in 2022. Spawn produced by hatcheries in 2022 and 2023 was sold to nurseries, traders and farmers (see 'Key Beneficiaries below). Most fish spawned in 2022 are now in grow-out ponds and their harvest will commence in late 2023.

Key Beneficiaries

Output 2: Dissemination of additional genetically improved carp

At the conclusion of The Project 38 private, 18 DoF, one BFRI and two educational institution hatcheries were known to maintain G3 rohu broodstock (Figure 7b).

Over the 2022 spawning season, 245 kg of spawn was produced by seven commercial hatcheries. Demand for this spawn by nurseries and grow-out farmers was high (Hamilton et al., 2023a), aided by the fact that on-farm trial results of the G3 multiplier population (Output 1) were obtained and disseminated during to the 2022 spawning season (see 'How the scientific results were disseminated' below).

The number of farmers supplied with fish spawned by commercial hatcheries in 2022 was estimated by i) monitoring seed sales to nurseries and farmers by hatcheries, and ii) monitoring sales by a representative sample of nurseries to farmers, and iii) extrapolating across all nurseries (Table 5). Spawn produced in 2022 was sold by hatcheries to 65 farmers and 104 nurseries. By the conclusion of The Project, these 104 nurseries had sold seed to approximately 3017 farmers (Table 5). This estimate is likely to be conservative, as some farmers act as seed traders and on-sell their fish to additional farmers. Furthermore, at the conclusion of The Project, nurseries retained some 2022 stock for future sale (Table 5).

In 2023, spawning and sales of G3 rohu commenced in April and continued until August. By the conclusion of The Project, 13 hatcheries had produced 2826 kg of G3 rohu spawn. Assuming 15 farmers are supplied for each kg of spawn produced (as in 2022; Table 5), 42,390 farmers are expected to use G3 rohu spawned in 2023. It is anticipated that G3 rohu spawn production will increase in 2024 and beyond as demand for the product increases, the number of mature broodstock increases and the size of individual broodstock increases.

Table 5. Inputs used to estimate the total number of farmers supplied with G3 rohu from the 2022 commercial spawning.

Number of farmers supplied with G3 rohu seed by the 22 sampled nurseries	432
G3 rohu spawn stocked by the sampled nurseries (kg)	24.9
Assumed portion of total seed sold by 30 June 2023 by the sampled nurseries	0.95
Estimated number of farmers supplied by each kg of spawn	18.3
Volume of spawn stocked by all 104 nurseries (kg)	173.9
Assumed portion of total seed sold by 30 June 2023 by all nurseries	0.95
Estimated number of farmers supplied with G3 rohu seed by all nurseries	3,017
Number of farmers supplied with G3 rohu seed directly from hatcheries	65
Total number of farmers supplied with G3 rohu by 30 June 2023	**3,082**

Output 5: Practical manuals, tools, and training activities

Two hatchery training workshops on the Management and Marketing of Genetically Improved Carp were conducted, attended by 60 men and 8 women (Oct 2022). Participants were provided with a manual, comprised of the presented slides translated into Bangla (Hamilton et al., 2022d)

How the scientific results were disseminated

Output 1: Performance analysis (on-farm trials)

Results of on-farm trials were disseminated as a peer-reviewed paper (Hamilton et al., 2022b); a presentation at the International Conference on Sustainable Fisheries, Sylhet, Bangladesh (Hamilton et al., 2022c); a Feed the Future Fish Innovation Lab success story (Hamilton and Yeasin, 2021) and news media articles:

- Dhaka Tribune: https://www.dhakatribune.com/bangladesh/2022/06/21/better-bred-rohu-ready-for-farming
- bdnews24: https://bdnews24.com/economy/2022/06/19/worldfish-says-its-g3-rohu-grows-30pc-faster-than
- Daily Star: https://www.thedailystar.net/star-health/news/worldfish-invented-g-3-rohu-grows-about-30-cent-more-conventional-one-3039666
- Bangla Insider: https://www.banglainsider.com/inside-bangladesh/114081

Output 2: Dissemination of additional genetically improved carp

Dissemination of additional genetically improved carp was publicised as a Feed the Future Fish Innovation Lab success story (Hamilton et al., 2023a), through a workshop held at BFRI headquarters in Mymensingh attended by 110 people (excluding WorldFish staff) and in the Bangladeshi media:

- Business Standard: https://www.tbsnews.net/bangladesh/worldfish-g3-rohu-handed-over-bangladesh-fisheries-research-institute-679266

- Daily Sun: https://www.daily-sun.com/printversion/details/705128/Fish-production-to-reach-8.6m-tonnes-by-2041
- Daily Samakal: https://samakal.com/whole-country/article/2308188575
- Dhaka Post: https://www.dhakapost.com/country/214176
- Bijoy TV: https://www.facebook.com/watch/?extid=WA-UNK-UNK-UNK-AN_GK0T-GK1C&mibextid=iCRzhm&v=675536377812853
- RTV: https://www.facebook.com/watch/?extid=WA-UNK-UNK-UNK-AN_GK0T-GK1C&mibextid=iCRzhm&v=9706551552749143
- Bangla Vision
- MY TV

Additional dissemination activities were funded from resources outside The Project. These included an information leaflet provided to hatcheries and other stakeholders (https://hdl.handle.net/20.500.12348/5429), three field days at on-farm trial sites (May 2022), seven hatchery seminars across seven districts – Jashore, Khulna, Barishal, Rajshahi, Rangpur, Bandarban, and Cox's Bazar – attended by 305 men and 33 women (Sept 2022) and a workshop at the time of handover of G3 rohu multiplier fish to the Department of Fisheries:

- Prothom Alo: https://www.prothomalo.com/bangladesh/ueprv6r0nl; https://en.prothomalo.com/bangladesh/qgetsthznv
- TBS news: https://www.tbsnews.net/economy/corporates/department-fisheries-receives-worldfish-rapidly-growing-g3-rohu-629114
- Jugantor: https://www.jugantor.com/corporate-news/672979

- Bonikbarta: https://bonikbarta.net/home/news_description/339908
- Channel I: https://drive.google.com/file/d/13TXfLz9IJMJuGXXiNnOFf0WwBm1vLwz6/view?usp=drivesdk
- News 24: https://drive.google.com/file/d/1DwDydGWlpPXRrX92wapI2zHdOqretgF0/view?usp=drivesdk
- RTV: https://drive.google.com/file/d/1Xc-Ubdw9Cukjbyh6FfAAubwvtZ2olj-o/view?usp=drivesdk
- Bangla TV: https://drive.google.com/file/d/1PieNEnOFaiqtqd_JmspGrmRny4X8CG4H/view?usp=drivesdk
- Ananda TV: https://drive.google.com/file/d/102eoZpb_ghSW5YIVrzaDUlFCKovWKJc_/view?usp=drivesdk
- Bijoy TV: https://drive.google.com/file/d/1fRqTVyoxWTXetwj4cXeNblqmMWd3Myux/view?usp=drivesdk

Output 3: New generations of improved carps

The new generations of improved carps were described in a presentation at a Feed the Future Fish Innovation Lab project meeting Mymensingh, Bangladesh and a presentation at Aquaculture America 2023, New Orleans, USA (Hamilton et al., 2023c).

Output 4: A cost-effective pedigree assignment tool

Results from Output 4 will be submitted to peer-reviewed journals for publication including Hamilton et al. (in prep).

Output 5: Practical manuals, tools, and training activities

Results from Output 5 were disseminated as a manual, comprised of the slides presented at training workshops, in English and Bangla (Hamilton et al., 2022d)

Output 6: A cryopreserved sperm repository strategy

Results from Output 6 were disseminated as a publicly-available report (Bodenstein et al., 2023)

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Appendices

Appendix 1. The WorldFish Carp Genetic Improvement Team

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