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A cryopreserved sperm repository strategy for WorldFish genetically improved carp

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Prof. Mostafa Ali Reza Hossain
Prof. Hossain passed away prior to the completion of this report. His knowledge, experience and approachable personality are missed by the co-authors and his passing represents an exceptional loss for fisheries and aquaculture research, and teaching, in Bangladesh.

About WorldFish
WorldFish is a nonprofit research and innovation institution that creates, advances and translates scientific research on aquatic food systems into scalable solutions with transformational impact on human well-being and the environment. Our research data, evidence and insights shape better practices, policies and investment decisions for sustainable development in low- and middle-income countries.

We have a global presence across 20 countries in Asia, Africa and the Pacific with 460 staff of 30 nationalities deployed where the greatest sustainable development challenges can be addressed through holistic aquatic food systems solutions.

Our research and innovation work spans climate change, food security and nutrition, sustainable fisheries and aquaculture, the blue economy and ocean governance, One Health, genetics and AgriTech, and it integrates evidence and perspectives on gender, youth and social inclusion. Our approach empowers people for change over the long term: research excellence and engagement with national and international partners are at the heart of our efforts to set new agendas, build capacities and support better decision-making on the critical issues of our times.

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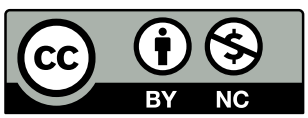

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List of acronyms

AGGRC	Aquatic Germplasm and Genetic Resources Center
BAU	Bangladesh Agricultural University
BFRI	Bangladesh Fisheries Research Institute
CGIP	Carp Genetic Improvement Program
DoF	Department of Fisheries
G3	Generation 3
LSUAC	Louisiana State University Agricultural Center

1. Introduction

Repositories of cryopreserved male gametes are a powerful means of preserving and advancing the genetic resources of commercial aquaculture species (Tiersch and Mazik, 2011; Torres and Tiersch, 2018). Germplasm repositories allow storage of gametes for long periods and provide a readily accessed source of genetic material that is easily transported. Repository storage has been used in aquaculture to avoid the loss of genetically significant populations (e.g., improved strains) and individuals, due to disease or natural disasters (Hu et al., 2011), and as an alternative to maintaining live animals thus freeing up space and other resources. Repository storage has been used in genetic-improvement programs to store genetic material from multiple generations to allow genetic change to be monitored and quantified across generations (Næve et al., 2022). Repositories can also facilitate genetic transfer among advanced-generation breeding populations and dissemination of genetically improved germplasm into terminal grow-out populations (Torres and Tiersch, 2018). For example, in terrestrial species, repository storage and artificial insemination have led to rapid and large-scale dissemination and exchange of genetically improved germplasm in the beef and dairy industries (Curry, 2000).

WorldFish manages family-based genetic improvement programs in Bangladesh for three significant aquaculture species, catla (*Catla catla*) (Hamilton et al., 2019a), rohu (*Labeo rohita*) (Hamilton et al., 2019b; Hamilton et al., 2022a) and silver carp (*Hypophthalmichthys molitrix*) (Hamilton et al., 2021). Activities of the WorldFish Carp Genetic Improvement Program (CGIP) are undertaken at a hatchery, grow-out, and broodstock rearing facility near Jashore, Bangladesh. Living backup populations of families are also maintained in the north and northwest of the country. WorldFish does not currently maintain a backup repository of cryopreserved gametes.

In 2020/2021, WorldFish disseminated genetically improved rohu multiplier populations – comprised of high ranking Generation 3 (G3) families – to commercial hatcheries (Hamilton et al., 2022b). These populations were supplied as hatchlings (i.e., without the use of cryopreserved gametes) to be grown into mature broodstock by the hatcheries. In 2022, the first sales of WorldFish G3 Rohu by these hatcheries took place. WorldFish took steps to begin repository development the same year by collaborating with the Aquatic Germplasm and Genetic Resources Center (located in Baton Rouge, LA, USA).

The collaboration utilized industrial engineering tools, such as relationship maps, to outline a repository network. Relationship maps are a common tool used in industrial engineering. They employ diagrams that can encompass multiple facilities and illustrate the interactions among suppliers, producers, and customers (Apaiah & Hendrix, 2005; Emelogu et al., 2019). With relationship maps the members of a system and their contributions to that system, such as the members of a germplasm repository network for carp in Bangladesh, are illustrated to aid in planning and implementation (Bodenstein et al., 2022).

This report identifies key stakeholders and pathways for the use of cryopreserved sperm repository technology for carp genetic improvement and dissemination in Bangladesh. Relationship mapping has been used to delineate relationships among suppliers, producers, and customers and their facilities.

2. Strategy development for sperm repositories

Strategy development was broken into five phases. Each phase comprised a videoconference, one-on-one communication between key participants and/or email correspondence.

- Phase 1. Defining the approach to strategy development
- Phase 2. Gathering information
- Phase 3. Real-time relationship map development
- Phase 4. Refinement of relationship maps
- Phase 5. Conclusions from the perspective of WorldFish

Phase 1. Defining the approach to strategy development

Objectives

- Define the approach to strategy development

Videoconference

- A videoconference was held on the 2 November 2022 attended by Sarah Bodenstein and Terrence Tiersch (AGGRC); Mostafa Hossain (BAU); and Matthew Hamilton (WorldFish).

Outcomes

- Strategy development will initially focus on the development of relationship maps, primarily in consultation with WorldFish staff.
- Key WorldFish participants were identified: Matthew Hamilton, Mohammed Yeasin, Md. Masud Akhter, Trọng Quốc Trịnh, Mahirah Mahmuddin.
- A list of specific questions to inform the development of relationship maps will be developed by Sarah Bodenstein prior to engaging with WorldFish staff.

Phase 2. Gathering information

Objectives

- Information gathering to inform the development of relationship maps

Videoconference

- A videoconference was held on the 14 November 2022 attended by Sarah Bodenstein (AGGRC); Mostafa Hossain (BAU); and Matthew Hamilton, Mohammed Yeasin, Md. Masud Akhter, Trọng Quốc Trịnh and Mahirah Mahmuddin (WorldFish)

Outcomes

- Specific questions were addressed:
  1. What are the goals of cryopreservation?
     i. Backup and safeguard carp WorldFish carp genetic resources in case of emergency.
     ii. Store male gametes to allow validation of genetic gains in the CGIP across generations (Næve et al., 2022).
     iii. Potentially overcome issues with spawning asynchronicity in the CGIP.
     iv. Potentially backup rare, unique or domesticated genetic resources from outside the CGIP.
     v. Potentially facilitate transfer of genetically improved carp genetics to hatcheries – in certain circumstances frozen sperm may be more effective than transporting fry (e.g., if transporting over long distances, or where biosecurity is an issue).

  2. What species will be housed in the repository? Will this change over time?
     i. WorldFish currently manages genetic improvement programs for catla *(Catla catla*); rohu (*Labeo rohita*) and silver carp (*Hypophthalmichthys molitrix*).
     ii. Additional carp species may be considered in the future (e.g., mrigal; *Cirrhinus cirrhosis*) but there are no immediate plans to expand the WorldFish CGIP.
     iii. Some consideration should be given to obtaining samples from rare, unique or important domesticated populations of carp species in Bangladesh.
     iv. If a nation-wide germplasm repository and dissemination system is developed, additional species would be included.

  3. What data should be collected?
     i. Dates
     ii. Names of technicians
     iii. Cryopreservation and storage procedures and reagents
     iv. Sperm parameters, and type and time of analysis

  4. Does a database currently exist? If not, how will a database be created?
     i. WorldFish CGIP genetic and phenotypic data is currently maintained within the WorldFish CGIP data management system (Hamilton,

2021)
   ii. Data pertaining to cryopreservation procedures and sperm parameters must be maintained. Off-the-shelf management systems exist that may be appropriate (e.g. BioEase™) for this purpose.
   iii. The same system should be used for WorldFish tilapia and carp cryopreservation data.
   iv. All carp cryopreservation data must be linked (using individual fish identifiers) to the WorldFish CGIP data management system.

5. Where will the repository be located?
   i. WorldFish manages a repository in Malaysia but this is unlikely to be suitable for the WorldFish CGIP given logistic and legal issues association with the transfer of genetic material across international borders.
   ii. WorldFish may decide to maintain its own repository in Bangladesh (most likely at Talbaria but possibly in Dhaka or Jashore). This would require investment in facilities, maintenance, and training.
   iii. Maintenance of primary or secondary repositories by external private, public, university, NGOs entities with existing repositories housing fish or terrestrial animal germplasm may be beneficial. This approach would require engagement with reliable, experienced, and trusted partners.

6. Should there be multiple repositories?
   i. Ideally yes, to provide redundancy in case of failure of one repository.
   ii. Relative risk, logistical considerations, budget considerations, and the availability of appropriate external repository facilities would need to be considered.

7. At what scale will the repositories need to process and distribute samples?
   i. Only small scale would be required to meet most goals of cryopreservation (Question 1, above). Samples should be collected at the time of each spawing (currently 6 spawning sessions are used to produce ~60 families each per year) so as to ensure the most recent generation of all WorldFish CGIP species and lines are maintained.
   ii. Larger scale would be required if cryopreserved sperm were to be shipped to hatcheries for dissemination.

8. How will samples be disseminated to the community?
   i. No dissemination to the community would be required, if acting as a backup or source of germplasm for internal WorldFish purposes.
   ii. If adopted for dissemination, this would involve shipment of cryopreserved sperm to hatcheries (not farmers).

9. How will repositories interact with the farmers (and other community members) besides providing samples?
   i. Repositories would not engage directly with farmers.

10. What samples will be shipped? Sperm, eggs, juvenile fish, etc.

i. WorldFish currently disseminates genetically improved germplasm to hatcheries as hatchlings. The distribution of hatchings is low-tech, cheap, effective, and allows dissemination of genetic contributions from genetically improved male and female parents over large distances.
ii. The transport of juvenile and adult fish is also possible within Bangladesh but more expensive and difficult than transporting hatchlings. WorldFish has recently assisted in the transfer of mature genetically improved broodstock among hatcheries.
iii. In the context of cryopreservation, only sperm can be frozen and stored. The preservation of eggs is not possible using existing technologies.

11. What kind of training needs to be provided to farmers and repository centers?
    i. Training of farmers will not be necessary, as they will purchase seed from hatcheries or nurseries.
    ii. If housing its own repository, WorldFish staff in Bangladesh have limited experience in the collection, cryopreservation, and analysis of samples and repository maintenance. Substantial training will be necessary.
    iii. Training of hatcheries in the use of cryopreserved sperm has commenced under the Fish Innovation Lab funded "Cryogenic sperm banking of Indian major carps (*Catla catla*, *Labeo rohita* and *Cirrhinus cirrhosus*) and exotic carps (*Hypophthalmichthys molitrix*, *Hypophthalmichthys nobilis* and *Ctenopharyngodon idella*) for commercial seed production and brood-banking" project (Sarder et al., 2023).

12. What equipment should be used to freeze?
    i. To be determined, depending on the application model implemented.

13. Are there currently people available to begin repository development?
    i. There is limited experience within the WorldFish team in Bangladesh
    ii. WorldFish currently maintains a repository for tilapia at its Malaysian headquarters and so there are highly-trained staff within the organisation that may be able to provide further training to Bangladesh-based staff.
    iii. Repositories exist for carp at Bangladesh Agricultural University (BAU) and for terrestrial species in other institutions in Bangladesh.

- Possible parties involved in a Bangladeshi carp repository network were identified as: WorldFish, Universities (e.g., BAU), private hatcheries, fisheries government agencies (i.e., Department of Fisheries, DoF, and the Bangladesh Fisheries Research Institute, BFRI), and external cryopreservation facilities.

- Preliminary relationship maps were developed by Sarah Bodenstein, to be refined in Phase 3.

Phase 3. Real-time relationship map development

Objectives
- Develop relationship maps

Videoconference
- A videoconference was held on 29 November 2022 attended by Sarah Bodenstein (AGGRC); and Matthew Hamilton, Mohammed Yeasin, Md. Masud Akhter, Trọng Quốc Trịnh and Mahirah Mahmuddin (WorldFish). Mostafa Hossain and Md. Rafiqul Sarder (BAU) were unable to attend.

Outcomes
- Relationship maps were developed in real-time during the course of the videoconference (Annex 1; Figures A1 and A2) and later refined.
- Protocols and systems developed as part of the Fish Innovation Lab 'sperm banking' project – Cryogenic sperm banking of Indian major carps (*Catla catla*, *Labeo rohita* and *Cirrhinus cirrhosus*) and exotic carps (*Hypophthalmichthys molitrix*, *Hypophthalmichthys nobilis* and *Ctenopharyngodon idella*) for commercial seed production and brood-banking (Sarder et al., 2023) – should be implemented where appropriate.
- Different relationship maps are required depending on the objectives of cryopreservation:
    1. to cryopreserve male parents of families produced in the WorldFish CGIP to safeguard WorldFish genetic resources.
        i. WorldFish would maintain its own repository and/or contract an external repository facility (non-fisheries public agencies, private or NGO) to maintain cryopreserved samples.
        ii. The sample collection would occur at the WorldFish hatchery, near Jashore.
        iii. WorldFish would aim to freeze samples from all male parents of full-sibling families produced at the hatchery each year (~360 samples).
        iv. WorldFish would inform the relevant government agencies in Bangladesh about their cryopreservation activities.

    2. to develop a nationwide germplasm repository and dissemination system for carp.
        i. WorldFish would maintain its own repository for its own germplasm.
        ii. External repository facilities (non-fisheries public agencies, private, university, or NGO) may provide cryopreservation services to external parties, including WorldFish.
        iii. Fisheries government agencies (i.e., the Department of Fisheries, DoF, and the Bangladesh Fisheries Research Institute, BFRI) do not currently maintain cryopreservation facilities. These agencies would have a role in approving and endorsing new technology and approaches to dissemination of cryopreserved germplasm. They would also have an ongoing role in seed production, training, and information gathering and reporting relating to cryopreserved

germplasm.

iv. WorldFish, universities, hatcheries and government agencies would have a role in optimising the production of high-quality seed (i.e., genetically improved, and not inbred) from cryopreserved germplasm. Ongoing research cooperation would be required to:
   - optimise freezing, storage, and dissemination protocols,
   - compare performance (among populations, strains, lines, and generations), and
   - assess the impact of cryopreservation.

Phase 4. Refinement of relationship maps

Objectives
- Refine relationship maps.

Videoconference
- Multiple informal videoconferences.

Outcomes
- Relationship maps developed in real-time in the Phase 3 videoconference were refined by Sarah Bodenstein and WorldFish staff (Figures 1 and 2).

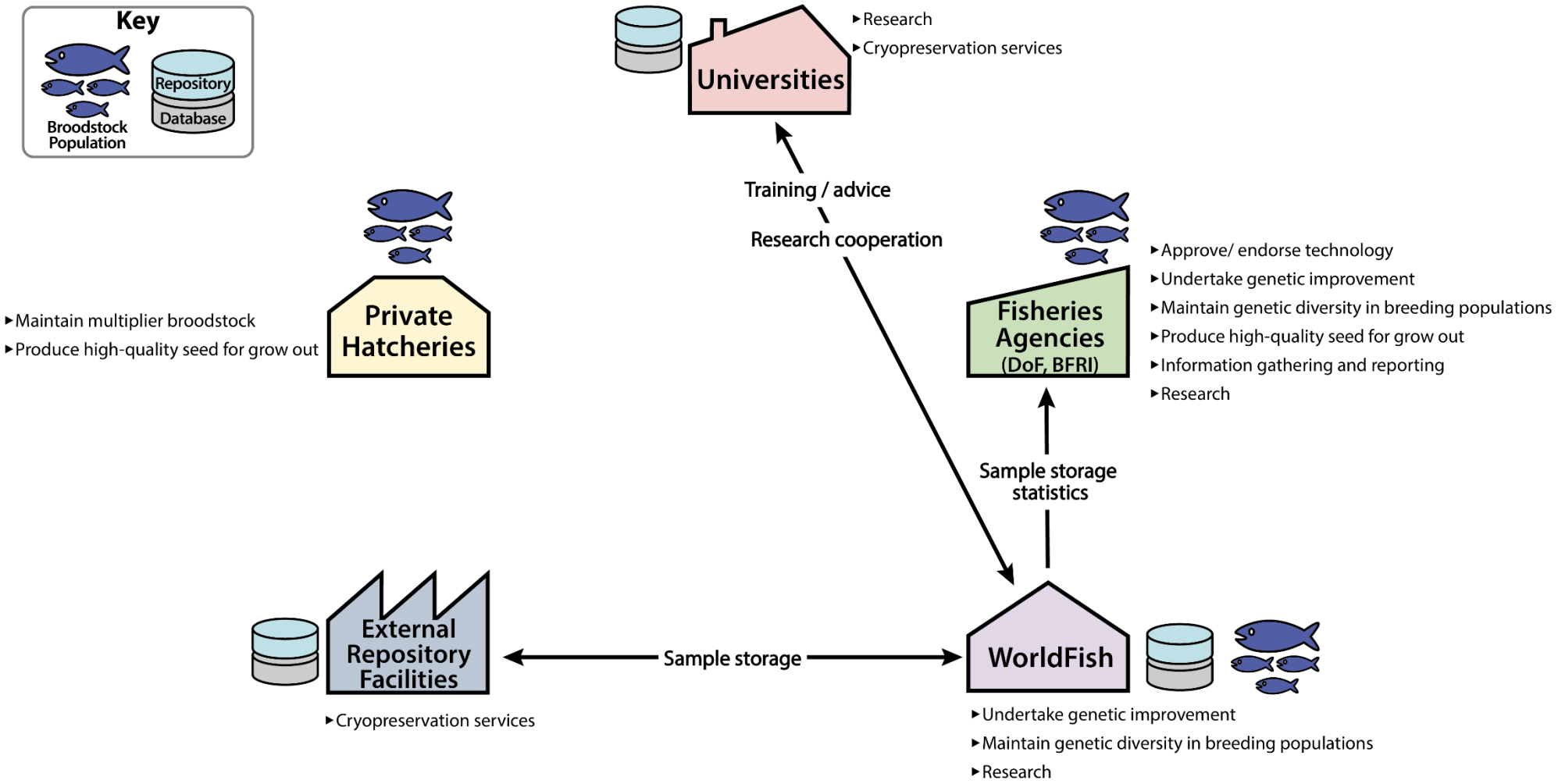

Figure 1. Relationship map outlining the interactions among entities involved in the cryopreservation of male parents of families produced in the WorldFish Carp Genetic Improvement Program, to safeguard WorldFish genetic resources.

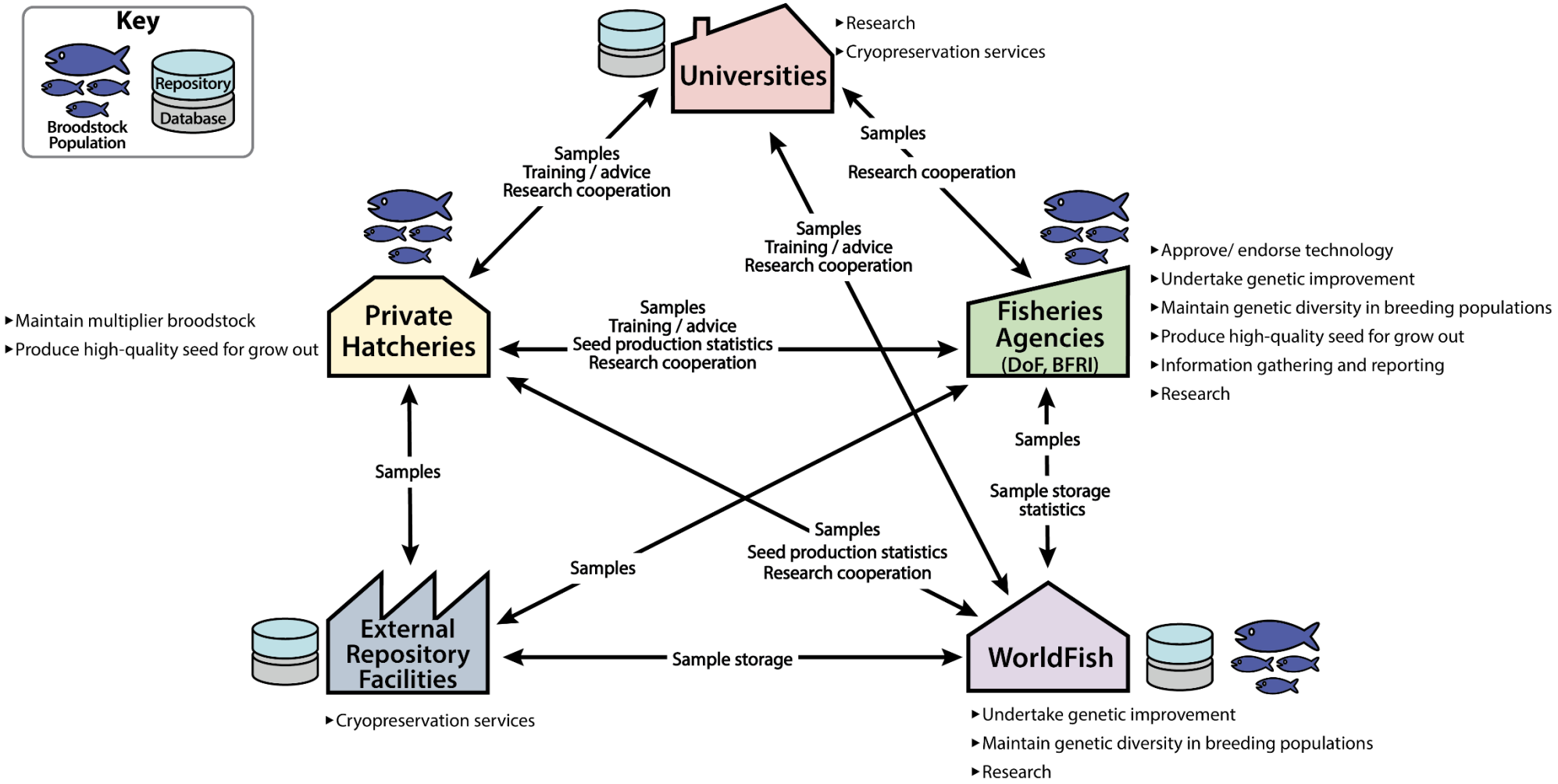

Figure 2. Relationship map outlining the interactions among the members of a nationwide germplasm repository system for carp.

Phase 5. Conclusions from the perspective of WorldFish

Objectives

- Document conclusions from the perspective of WorldFish.

Videoconference

- Multiple informal videoconferences.

Outcomes

1. The viability and desirability of developing a WorldFish carp germplasm repository should be judged against:
    - Relative risk and the perceived risk of catastrophic loss of all living backup populations,
    - the value of cryopreserved sperm in the event of catastrophic loss (e.g., disease outbreak),
    - cold-chain logistics,
    - budget considerations including establishment and running costs,
    - the availability of trained staff, and
    - the availability of appropriate external repository facilities.

2. WorldFish should continue to maintain living backup populations, in parallel with any cryopreserved germplasm repository.

3. If a WorldFish carp germplasm repository is to be established, consideration should be given to obtaining backup samples from existing rare, unique, and important domesticated populations of carp species in Bangladesh.

4. When developing relationship maps for a nationwide germplasm repository system for carp, no compelling current reason to utilise cryopreserved sperm to disseminate WorldFish genetically improved germplasm was identified. Accordingly, WorldFish should continue to disseminate genetically improved germplasm as hatchlings to hatcheries – this approach is low-tech, cheap, effective, and allows the dissemination of genetic contributions from genetically improved male and female parents across Bangladesh.

5. If a nationwide germplasm repository system for carp is developed, WorldFish would need to consider the benefits of engaging with a wider network against the costs and risks (e.g., loss of control of genetically improved germplasm), particularly in the context of any future commercialisation of WorldFish genetically improved carp.

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Annex 1. Draft relationship maps

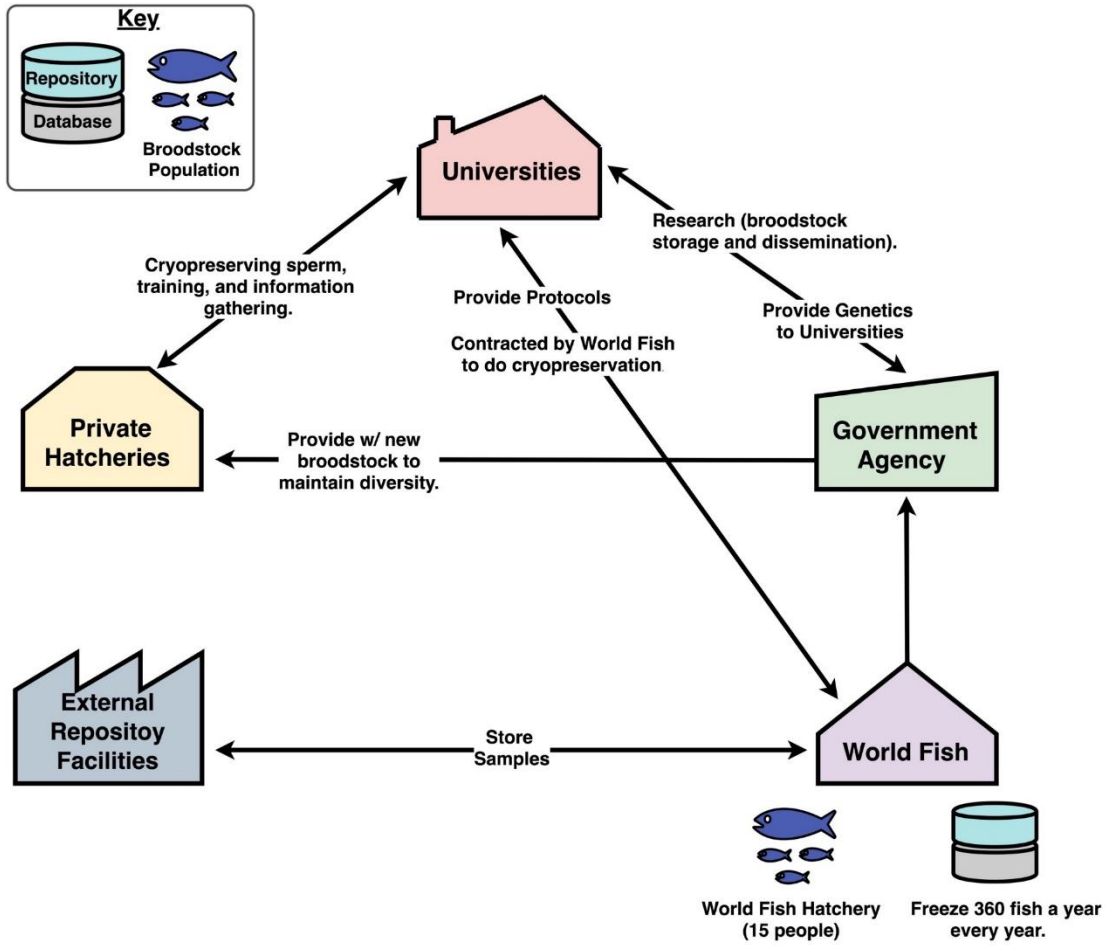

Figure A1. Draft relationship map outlining the interactions among entities involved in the cryopreservation of male parents of families produced in the WorldFish Carp Genetic Improvement Program, to safeguard WorldFish genetic resources.

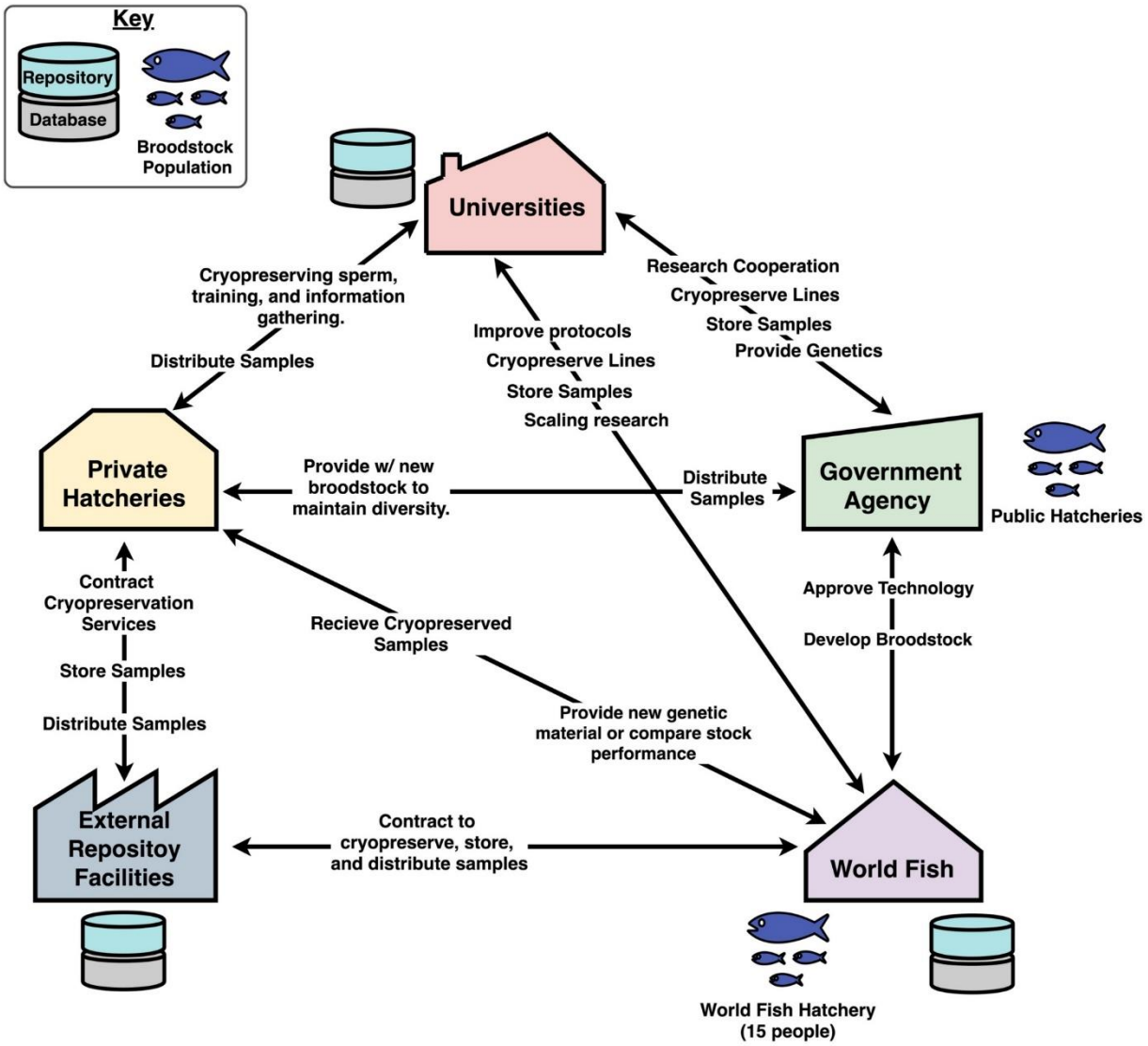

Figure A2. Draft relationship map outlining interactions among the members of a nationwide germplasm repository system for carp.

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