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GIFT transfer risk management: Pathogen



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GIFT transfer risk management: Pathogen

Authors

J. Richard Arthur¹

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Contact

WorldFish Communications and Marketing Department, Jalan Batu Maung, Batu Maung, 11960 Bayan Lepas, Penang, Malaysia. Email: worldfishcenter@cgiar.org

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List of abbreviations

ALOP	appropriate level of protection
ALOR	acceptable level of risk
BIV	Bohle iridovirus
CEV	carp edema virus
EHNV	epizootic hematopoietic necrosis virus
EUS	epizootic ulcerative syndrome
FAO	Food and Agriculture Organization of the United Nations
F	Filian generation of fry produced from WorldFish broodstock
G	Generation of WorldFish broodstock
GIFT	Genetically Improved Farmed Tilapia
GON	Government of Nigeria
ICES	International Council for the Exploration of the Sea
ICLARM	International Center for Living Aquatic Resources Management
KHV	koi herpesvirus
IPNV	infectious pancreatic necrosis virus
IRA	import risk analysis
ISKNV	infectious spleen and kidney necrosis virus
LCDV	lymphocystis disease virus
NAQS	Nigeria Agricultural Quarantine Service
NBC	nucleus breeding center
OIE	World Organisation for Animal Health

PCR	polymerase chain reaction
PMP/AB	Progressive Management Pathway for Improving Aquaculture Biosecurity
PRA	pathogen risk analysis
PLOs	<i>Piscirickettsia</i> -like organisms
RGNNV	red grouper nervous necrosis virus
RSIV	red seabream iridovirus
SOPs	standard operating procedures
SPF	specific pathogen free
SPS Agreement	Sanitary and Phytosanitary Agreement
SVCV	spring viremia of carp virus
TAAD	transboundary aquatic animal disease
TiLV	tilapia lake virus
TiPV	tilapia parvovirus
TLEV	tilapia larval encephalitis virus
VER	viral encephalopathy and retinopathy
VNN	viral nervous necrosis
VNNV	viral nervous necrosis virus
WTO	World Trade Organization

Executive summary

In summary, the proposal to transfer Genetically Improved Farmed Tilapia (GIFT) to Nigeria is characterized by a number of risk management measures that WorldFish has already implemented. WorldFish has also proposed additional biosecurity measures in its transfer plan and in the draft of a prospectus for transfer risk management. This document recommends additional risk management measures that will help to ensure a low risk of pathogen introduction. While there are a number of serious pathogens of Nile tilapia, the history of the GIFT stock, the risk management measures that WorldFish has already implemented or will implement, and the additional measures proposed in this document are sufficient to remove all of these pathogens from consideration as potential hazards.

This risk analysis examines the pathogen risks associated with the proposed importation of fry of GIFT, a strain of Nile tilapia (*Oreochromis niloticus*) developed by WorldFish that is characterized by its improved growth characteristics, to Nigeria for aquaculture development. It is one of three separate but related expert risk assessments commissioned by WorldFish Malaysia (1) to evaluate the genetic, ecological and pathogen risks associated with the proposed transfer and (2) to outline a risk management plan.

Only a few dozen comprehensive risk analyses have been conducted globally on the pathogen risks posed by the introduction of a live aquatic animal for aquaculture development. This is the second such risk analysis conducted for the introduction or transfer of tilapia to the African continent and its commissioning by WorldFish, along with the associated genetic and ecological risk assessments, demonstrates a high level of social responsibility.

This analysis highlights the high level of risk management measures that WorldFish has either implemented or proposed. These include the following:

- Using fry derived from the GIFT breeding program broodstock (generation 17, G17), which has a known production and health history.
- Conducting diagnostic testing of G17 and the fry to be imported into Nigeria (G17–F1) to show that they are free from key pathogens.
- Upon arrival into Nigeria, placing of imported GIFT fry into a high-security quarantine facility.
- Monitoring and testing of GIFT fry during quarantine for key pathogens and for any cause of significant mortality.
- Releasing only of G17–F3 GIFT into nucleus breeding centers and subsequent diagnostic testing.
- Diagnostic testing of tilapia and catfish currently cultured in the facility in Nigeria for key pathogens.
- Establishing an expert risk management panel.
- Assembling an independent national scientific advisory team.

These risk management measures are among the biosecurity arrangements comprising world's best practice for the introduction or transfer of live aquatic animals.

This risk analysis considers 69 pathogens/pathogen groups (possible hazards) that have been reported globally from Nile tilapia. A number of these possible hazards are serious pathogens of Nile tilapia. However, this analysis suggests that the risk management measures proposed by WorldFish, further strengthened by the additional risk management measures outlined in this document, are likely to be sufficient to remove all these pathogens from consideration as hazards that could be released into the aquatic environment in Nigeria via the transfer of GIFT fry.

The additional risk management measures recommended in this document are placed into two categories: (1) actions that are essential for validating this risk assessment and (2) additional recommendations to be considered.

Essential actions

- **Monitor implementation of risk management measures.** As the risk assessment is highly dependent upon the actions proposed by WorldFish, monitoring systems should be established to ensure that all risk management measures are fully and effectively implemented.
- **Conduct diagnostic testing for additional pathogens.** Testing for viral nervous necrosis virus (VNNV), spring viremia of carp virus (SVCV), tilapia parvovirus (TiPV) and *Aquabirnavirus* should be included as part of the WorldFish protocols, as well as the health certification from the Government of Malaysia that will accompany the shipment of GIFT fry to Nigeria.
- **Ensure that the receiving facility in Nigeria meets all standards for high-security quarantine.** In preparation for transfer of GIFT, the receiving facility must remove all current stocks and disinfect all tanks, pipes, surfaces and fomites thoroughly, using an approved method. Specific standard operating procedures (SOPs) for the facility must be developed and put in place, and all staff must receive appropriate training to ensure that SOPs are strictly followed.
- **Examine GIFT broodstock for direct life-cycle parasites.** A few of the parasites that infect Nile tilapia, specifically *Gyrodactylus* and *Dactylogyrus*, may pose a significant risk to Nigeria, and a number of others are known to cause disease problems in aquaculture facilities. As such, the parent broodstock held in Penang should be subjected to full parasitological examination, as it may be possible, through appropriate treatment to minimize the chance that these pathogens will be transferred along with their hosts.
- **Develop a detailed contingency plan.** Serious exotic pathogens have escaped from similar high-biosecurity facilities. Thus WorldFish and the Government of Nigeria (GON) should develop a detailed contingency plan to deal with such an event, no matter how unlikely. This should include health monitoring of cultured stocks and diagnostic testing to determine the cause of any serious mortality events, as well as planning for efforts to restrict pathogen spread and, where possible, to implement eradication procedures.

Additional recommendations

- **Study the diseases of fish species near GIFT aquaculture facilities.** Baseline studies of the diseases of fish species in the vicinity of GIFT aquaculture facilities should be conducted, as such monitoring will help to detect any transfer of introduced exotic pathogens from GIFT to wild finfish populations.
- **Commission an expert review of testing protocols.** WorldFish may wish to have an independent expert in fish disease diagnostics review the proposed testing protocols for the final list of pathogens for which certification of GIFT fry will be conducted.
- **Stress test GIFT to reveal hidden pathogens.** Stress testing of broodstock and fry could be conducted to check for cryptic or unknown pathogens.
- **Organize a collaborative study of Nigerian fish pathogens.** WorldFish should consider developing an international project to conduct baseline surveys of key cultured species. Such studies should include viruses, bacteria and parasites.
- **Prepare a fish parasite checklist.** The preparation of a checklist of the parasites of Nigerian fish should be supported.
- **Assist in a decision on Nigeria's ALOP.** WorldFish should initiate discussions with the GON and relevant stakeholders to determine a national appropriate level of protection (ALOP). A national consultation involving the plant, terrestrial animal and aquatic animal health sectors could be convened to assist in reaching a consensus among stakeholders.
- **Develop a national strategy for aquatic animal health.** WorldFish could assist the GON to prepare its national strategy for aquatic animal health and participate in the Food and Agriculture Organization of the United Nations (FAO) Progressive Management Pathway for Improving Aquaculture Biosecurity (PMP/AB) program.
- **Implement measures to prevent future transfers of tilapia.** The GON should take all possible measures to prevent future transfers of tilapia of unknown health or genetic status from outside the national territory.

1. Introduction

1.1. Purpose

This document analyzes the pathogen risks associated with the proposed transfer of GIFT from Malaysia to Nigeria. GIFT is a fast-growing strain of Nile tilapia (*Oreochromis niloticus*) developed by WorldFish, formerly known as the International Center for Living Aquatic Resources Management (ICLARM). The Honorable Minister of Agriculture and Rural Development in Nigeria made the request to import GIFT into Nigeria to facilitate the country's national aquaculture development. This analysis is one of three separate but related expert studies that WorldFish has commissioned (a) to evaluate the possible (i) genetic, (ii) ecological and environmental and (iii) pathogen risks associated with the proposed transfer and (b) to develop associated risk management plans.

The details of the proposal are outlined in a strategic plan that WorldFish has developed for transferring GIFT from Malaysia to Nigeria, as well as the draft of a prospectus for transfer risk management. Briefly, using G17 GIFT as parents, swim-up fry (G17–F1) sourced from WorldFish's GIFT broodstock facility in Penang, Malaysia, will be transferred to a high-level biosecurity quarantine facility to be established in Nigeria's Ogun State through a WorldFish project. Following a successful quarantine, third generation fry (G17–F3) (non-sex reversed) will be moved to a nucleus breeding center (NBC) in Delta State and sex-reversed fry will be cultured in land-based and water-based systems (ponds and cages) in the two states. Subsequent generations of fry produced by NBCs will be made available to the private sector to help the GON establish a GIFT seed and grow-out industry.

1.2. Terms of reference

Terms of reference for the present consultancy are as follows:

The pathogen risk management plan will be developed by Dr. Richard Arthur from Canada. He will do the following:

- Examine and review the information provided by WorldFish on pathogen and disease aspects associated with the proposed transfer.
- Conduct, with the assistance of WorldFish (if requested), a detailed review of the relevant literature dealing with the pathogens and parasites of Nile tilapia.
- Follow best current practices for import risk analysis (IRA): in general, the methods outlined in FAO Fisheries and Aquaculture Technical Paper No. 519 (in particular, the paper by Arthur 2008), the IRA process as outlined in the Aquatic Animal Health Code of the World Organisation for Animal Health (OIE) (2019a) and the guidelines given in the International Council for the Exploration of the Sea's (ICES) Code of Practice for the Introductions and Transfers of Marine Organisms (2005).
- Assess both direct and indirect pathogen risks to the receiving environment that may result from the proposed transfer.
- Provide a document summarizing the results of the risk analysis and outlining a pathogen risk management plan, including recommended risk management measures, that could be implemented prior to, during and after transferring GIFT from Malaysia to Nigeria.

1.3. Background

Past experience has amply demonstrated that the international movement of live aquatic animals (fish, crustaceans and shellfish) of unknown or uncertain health status is a high-risk activity that has been responsible for the spread of many serious aquatic animal diseases to new geographical areas, often with

serious economic consequences.² It is clear that any proposal to transfer a new strain of any native species, such as Nile tilapia to Nigeria must include rigorous guarantees and risk management measures to ensure that the imported stock is free from serious transboundary aquatic animal diseases (TAADs).³

The range of pathogens and parasites infecting Nile tilapia is relatively well documented thanks to the global importance of this fish as an aquaculture species, which has generated hundreds of reports and publications. However, although Nile tilapia has been often introduced into new regions and countries for aquaculture development, there has been little consideration of the pathogens and parasites that could accompany them. Only one comprehensive IRA for this species can be found in the literature (i.e. Johnston 2008). Nile tilapia is susceptible to a number of untreatable serious diseases, mainly of viral etiology, several of which are listed by the OIE (2019a). The health status of Nile tilapia populations in Nigeria with regard to these serious pathogens has been little investigated. Thus a precautionary approach dictates that all possible measures should be taken to avoid their introduction into Nigeria through the use of appropriate biosecurity measures. One in particular is the repeated diagnostic testing of source broodstock and the fry to be transferred to demonstrate freedom from these pathogens (Section 7.1).

Countries considering either the introduction or transfer of live aquatic animals are recommended to follow the full ICES protocol for introductions and transfers of marine organisms (ICES 2005 and 2012). It should be emphasized that unlike the global shrimp culture industry, there exist no specific pathogen free (SPF) stocks of Nile tilapia that can be used to produce high health fry or juveniles originating from a production facility with guarantees that they are free from specific pathogens.



Photo credit: WorldFish

Genetically improved farmed tilapia (GIFT) in Jitra, Malaysia.

2. Commodity description

Table 1 defines the precise nature of the commodity to be transferred.

Species to be introduced:	<i>Oreochromis niloticus</i> (Nile tilapia), Genetically Improved Farmed Tilapia (GIFT) strain
Proposed date of importation:	June 2021
Life-cycle stage to be imported:	Fry only (G17–F1)
Importer:	Government of Nigeria
Exporter:	WorldFish Malaysia
Source:	WorldFish’s high-security GIFT broodstock facility in Penang, Malaysia
Proposed number of shipments:	1 (if the project is successful, subsequent shipment(s) may be needed to maintain stock quality)
Volume:	10,000 swim-up fry
Proposed destination:	A high-biosecurity quarantine facility to be established in Ogun State under a WorldFish project, with eventual release of progeny (G17–F3) into NBCs in Ogun and Delta states, followed by eventual release into the private sector for aquaculture development.

Table 1. Commodity description for the proposed transfer of Nile tilapia in Nigeria.

3. International and national context of the risk analysis

3.1. International context

Risk analysis is an internationally accepted standard method for assessing whether trade in a particular commodity, such as a live aquatic animal or its product, poses a significant risk to human, animal or plant health and, if so, what measures could be adopted to reduce that risk to an acceptable level. Several international factors have spurred the development of risk analysis. They include the liberalization of international trade through the General Agreement on Tariffs and Trade and the establishment of the World Trade Organization (WTO) and its Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement). WTO member countries are now required to use the risk analysis process as a means to justify any restrictions on international trade beyond those specified by the Aquatic Animal Health Code (OIE 2019a). These must be based on risks to human, animal or plant health (WTO 1994; Rodgers 2004).

3.1.1. Precautionary approach

The concept of the precautionary approach is widely used in fisheries management and elsewhere where governments must take action based on incomplete knowledge (Garcia 1996). The Code of Conduct for Responsible Fisheries, Section 7.5.1 (FAO 1995) states the following:

“States should apply the precautionary approach widely to conservation, management and exploitation of living aquatic resources in order to protect them and preserve the aquatic environment. The absence of adequate scientific information should not be used as a reason for postponing or failing to take conservation and management measures.”

In assessing potential pathogen-related risks associated with the proposed introduction or transfer of a live aquatic animal species, a precautionary approach requires that both the importing and exporting nations act responsibly and conservatively to avoid introducing potential “pest” species and spreading serious pathogens (Arthur et al. 2004).

Because WorldFish is a key global agency for advancing aquaculture, the proposed transfer of Nile tilapia to Nigeria should incorporate all feasible risk management measures and thus should exceed, to the extent possible, the minimal requirements of IRA. The lack of Nigerian expertise in key areas such as aquatic risk analysis, disease diagnosis and treatment, pathogen identification and biosecurity also places the burden on WorldFish to ensure a very low risk of disease transfer.

3.1.2. Previous risk analyses for tilapia

Only a few pathogen risk analyses have been conducted for the importation of tilapia or their products. These are summarized below:

- **Government of New Zealand, IRA for the importation of skinless boneless fillets of tilapia to New Zealand from China and Brazil** (Johnston 2008). This is the most comprehensive risk assessment conducted to date. Because of the nature of the product to be imported (fillets only), this study was able to immediately rule out many species of parasite that have been reported from tilapia species. It identified 13 pathogens of potential concern. These included several viruses (iridoviruses, aquatic birnaviruses), bacteria (*Aeromonas salmonicida*, *Flavobacterium* spp., *Streptococcus iniae*, *Edwardsiella* spp., intracellular bacteria, *Yersinia ruckeri*), parasites (*Henneguya* spp., digenean metacercaria, larval nematodes), and fungi (*Ichthyophonus hoferi* and *Aphanomyces invadans*). After further assessment, however, none of these potential hazards were identified as requiring specific risk management measures. In this instance, separating the fillets from the rest of the carcass was considered effective in removing the majority of organisms that might be present in the live animal.
- **IRA for the importation of Nile tilapia in South Africa** (Government of South Africa n.d.). This brief report focused mainly on potential ecological risks, particularly the risk of introduced *O. niloticus* hybridizing with the native *O. mossambicus*. No detailed pathogen risk assessment was conducted,

and import permits were issued on an ad hoc basis following negotiations between the South African competent authority and its counterpart in the exporting country (Dr. D. Huchzermeyer, personal communication, 2020). However, the report noted that “Caution should be taken to ensure that [pathogens] are not transferred to new bodies of water along with Nile tilapia that is intended for aquaculture.”

- **IRA for potential invasiveness of introduced GIFT tilapia to coastal marine ecosystems in Vanuatu** (Welch 2020). This study also focused on potential environmental risks due to a proposed introduction of GIFT. Although the potential for introducing pathogens was only briefly mentioned, the pathogen risk was considered “moderate.”
- **Tilapia lake virus (TiLV). Expert knowledge elicitation risk assessment** (FAO 2018). This study focused only on TiLV. The experts suggested that in terms of lower likelihood of entry, establishment and spread, and associated consequences, the risk of TiLV to Asia, Africa and South America was higher than that to the Pacific Island Countries and Territories and North America.
- **Preliminary risk assessment for tilapia lake virus (TiLV) to the USA** (USDA 2019). This study estimated that the risk of TiLV introduction to United States tilapia populations via the import of live tilapia and tilapia products was negligible for frozen tilapia fillets but high for imported tilapia fingerlings, germplasm and the associated shipping water. This is due to several factors: (a) the high degree of mortality when infection is present, (b) the lack of knowledge about how TiLV is spread, (c) the lack of regulations associated with importing tilapia fingerlings, and (d) the lack of a surveillance program and a response plan in the US should an outbreak occur.
- **Risk assessment of parasitic helminths between cultured and wild Nile tilapia** (Akoll et al. 2012). This study, conducted in Uganda, examined the potential risks posed by helminths infecting wild Nile tilapia to Nile tilapia cultured in cages and earthen ponds. The authors concluded that monogeneans are high-risk parasites, while heteroxenous helminths pose low to negligible threats to farmed fish.

3.2. National context

3.2.1. Relevant agreements and competent authority

The general framework for an IRA for live aquatic animals and their products is laid out in the OIE’s Aquatic Animal Health Code (OIE 2019a). In addition, the GON, as a member of both the OIE and the WTO, is obligated to follow OIE and WTO procedures. Nigeria’s delegate to the OIE is the chief veterinary officer, currently Dr. Olaniran Alabi, director of the Veterinary and Pest Control Services at the Ministry of Agriculture and Rural Development.

Although not obligatory to the GON, the ICES Code (2005 and 2012) has wide global acceptance. The code is considered the key framework for assessing proposals to introduce exotic species into new environments outside their native range or to transfer new strains of established species to countries where they are either native or previously introduced. Among other risk analysis frameworks, the ICES Code addresses the evaluation of potential genetic, ecology and pathogen risks associated with the transfer of aquatic organisms. As such, conforming with the recommendations of the code can be considered best practice when introducing new species for aquaculture development. WorldFish has prepared both a draft strategic plan and a prospectus for transferring GIFT to Nigeria that addresses the issues and concerns outlined in the ICES Code.

The Nigeria Agricultural Quarantine Service (NAQS), under the Federal Ministry of Agriculture and Rural Development, was created to harmonize plant, veterinary and aquatic resources (fisheries) quarantine in Nigeria. Its purpose is to promote and regulate sanitary (animal and fisheries health) and phytosanitary (plant health) measures in connection with importing and exporting agricultural products, with a view to minimizing the risk to the agricultural economy, food safety and the environment. Among other duties, NAQS is responsible for preventing the introduction, establishment and spread of animal and zoonotic diseases, including diseases of aquatic animals. NAQS undertakes emergency protocols to control or manage disease outbreaks in collaboration with key stakeholders. It also ensures that Nigeria’s agricultural exports meet international standards, including those of the OIE, the WTO’s

SPS Agreement, and SPS conditions set by importing countries. Its operations are guided by the enabling legislation enacted by the National Assembly and SPS regulations and schedules.⁴

3.2.2. Nigeria’s appropriate level of protection

The ALOP, also referred to as the acceptable level of risk (ALOR), is the level of protection that a country deems appropriate when establishing a sanitary or phytosanitary measure to protect human, animal or plant life or health within its territory (WTO 1994). As such, establishing an ALOP is a political decision, rather than a scientific one, and must be made at the highest level of government. Where no formal statement of ALOP exists, a country’s ALOP may often be defined by its practices in protecting its human, animal and plant life from hazards, as reflected in its legislation and other official documents, policies and procedures (Wilson 2000). The GON has apparently never formally declared a national ALOP. However, using the precautionary principle would dictate that in conducting the current pathogen risk

analysis (PRA) and recommending management measures to reduce pathogen risk, a conservative approach to protecting Nigeria’s aquatic animal health status should be followed. This means that a “high” ALOP should be applied. As such, the level of risk considered acceptable in this study should be characterized as “low” (AQIS 1999).

3.2.3. Nigeria’s river systems

Nigeria is blessed with abundant aquatic resources. The Niger River Basin is home to more than 100 million people of West and Central Africa and is the continent’s third-largest river system (Anderson et al. 2005). Nine countries comprise the Niger River Basin: Benin, Burkina Faso, Cameroon, Chad, Cote d’Ivoire, Guinea, Mali, Niger and Nigeria (Figure 1). Within Nigeria, there are 11 distinct river basins, almost all of which drain into the Niger River (Amasu 1981; Ezenweani 2017). Because of this, ill-considered introductions or transfers of live aquatic animals into Nigeria have the potential to impact ecosystems and human populations over a large area of West Africa.



Source: Golitzen KG, Andersen I, Dione O and Jarosewich-Holder M. 2005. *The Niger River Basin: A Vision for Sustainable Management. Directions in Development.* Washington, DC: World Bank. © World Bank. <https://openknowledge.worldbank.org/handle/10986/7397> License: CC BY 3.0 IGO.

Figure 1. The Niger River Basin in West Africa.

4. Risk analysis methods

4.1. General approach

The general approach used in the PRA follows that outlined by the AFFA (2001), Arthur et al. (2004 and 2009), Arthur and Bondad-Reantaso (2012) and the OIE (2019a).

The risk analysis process as outlined by the OIE (2019a) includes four components: hazard identification, risk assessment, risk management and risk communication. Risk communication will be the responsibility of WorldFish and the GON and is expected to include extensive stakeholder consultation.

4.2. Hazard identification

A hazard is any pathogenic agent that could produce adverse consequences upon importing a commodity (a live aquatic animal or its product), while hazard identification is the process of identifying pathogens that could potentially be introduced in the commodity considered for importation. As the potential pathogens of Nile tilapia, on a global basis, number in the hundreds, this risk analysis does not strictly follow the IRA framework as outlined by the OIE (2019a). Instead, it addresses hazard identification in the following fashion:

- “Possible hazards” (pathogens and parasites recorded from Nile tilapia globally) are listed and then evaluated against a set of criteria to determine those taxa that should be considered “potential hazards” (pathogens that pose a real risk to Nigeria). It is important to note that this initial step does not take into consideration the risk management measures in progress or proposed by WorldFish. However, the WorldFish risk management measures are taken into consideration in the subsequent steps of the risk assessment process.
- The potential pathogens are then individually evaluated using a stepwise pathways approach to estimate the likelihood of their entry into Nigeria (“entry assessment”).
- If the estimated likelihood of entry is “non-negligible,” then the likelihood of exposure is estimated (exposure assessment).

- If both of these estimates are non-negligible, then they are combined to give an estimate of the “likelihood of entry and exposure.”
- If this combined likelihood estimate is non-negligible, then the magnitude of the consequence of entry and exposure is estimated.
- If the estimate of consequence is non-negligible, then the two non-negligible estimates are combined to estimate the total risk posed by the hazard (risk = likelihood x consequence).
- The estimate of total risk posed by the hazard is then compared with the ALOP suggested for Nigeria to determine if the risk is acceptable.
- If the risk is unacceptable, then, where possible, additional risk management measures are suggested that will reduce the risk to an acceptable level.

Such an approach to IRA has been successfully used to minimize the risks associated with the introduction of penaeid shrimp species to the Kingdom of Saudi Arabia (Arthur et al. 2012) and the Sultanate of Oman (Aranguren and Arthur 2018a and 2018b). This approach has been accepted by independent expert review, stakeholder consultation and the respective competent authorities.

4.3. Assumptions of the risk analysis

This risk analysis is based on the following assumptions:

- As the source of the GIFT fry, WorldFish’s broodstock facility meets the standards for a high-security quarantine facility.⁵ In particular, it is assumed that standards of biosecurity are such that no potential pathogens have been able to enter the facility from the external environment (i.e. pathogens present in the fauna and aquatic environments of Malaysia have been excluded. As a result, the only pathogens that could be present in the facility

are those that may have accompanied the original stocks of Nile tilapia that were used to develop the GIFT strain. This assumption allows the risk analysis to remove from consideration:

- all possible pathogens having a life cycle that involves an obligatory alternate or intermediate host.
- the possibility that the broodstock has acquired new pathogens or parasites infecting the fish fauna of Malaysia through the facility's water source or introduced via facility staff and their various activities.
- Additionally, this risk analysis takes a precautionary approach, by assuming that pathogens and parasites that are not reported as present in Nigeria and that are not known to be ubiquitous are to be considered absent from the country. The status of knowledge on pathogens and parasites of the Nigerian cichlids is summarized in Section 5.3.

4.4. Pathogen groups excluded from analysis

Table 2 lists the major taxonomic categories of potential pathogens infecting Nile tilapia and the nature of their life cycles (direct or indirect). The GIFT fry to be imported originate from broodstocks that have been cultured for many generations under conditions that would preclude infection by parasites with indirect life cycles. As such, the Myxozoa, Digenea, Cestoda, Nematoda, Acanthocephala, Pentastomida and some Protista have been excluded from further consideration.

4.5. Risk management

Risk management is the process of evaluating the estimated risk to determine if it is significant to the importing country, and if it is, of identifying, documenting and implementing measures that can be applied to reduce the level of risk to an acceptable level as expressed (explicitly or implicitly) in the country's ALOP. Risk management measures for a given hazard (risk mitigation) are only considered when the estimated level of risk for the hazard exceeds the country's ALOR. The level of unmitigated risk, the ALOR and the individual nature of the hazard will determine what risk management measures, if any, can be applied to reduce the risk to an acceptable level.

In this risk analysis, WorldFish has initiated or proposed extensive risk management measures to reduce the likelihood that serious pathogens will be introduced and become established in Nigeria. The risk analysis therefore takes these measures into consideration during the hazard evaluation process and will only suggest additional risk management measures should the proposed measures be insufficient to reduce the risk posed by a hazard to an acceptable level.

4.6. Terminology

4.6.1. Terms used to describe the probability of an event occurring

This risk analysis follows a five-category system to assess whether an adverse event is likely to occur:

1. High: very likely to occur
2. Moderate: an even probability of occurring
3. Low: unlikely to occur
4. Very low: very unlikely to occur
5. Negligible: almost certainly not to occur.

4.6.2. Terms used to describe the consequences of an event occurring

The terms used to describe the consequences of an adverse event occurring follow those outlined by AQIS (1999):

- Catastrophic: Establishment of disease would be expected to cause significant economic harm at a national level and/or cause serious and irreversible harm to the environment.
- High: Establishment of disease would have serious biological consequences (e.g. such as high mortality or morbidity) and would not be amenable to control or eradication. Such diseases could significantly harm economic performance at an industry level and/or may cause serious harm to the environment.
- Moderate: Establishment of disease would have less pronounced biological consequences and may be amenable to control or eradication. Such diseases could harm economic performance at an industry level and/or cause some environmental effects, which would not be serious or irreversible.

Taxonomic group	Type of life cycle	Considered in hazard identification?	Remarks
Viruses	Direct	Yes	<p>These may be transferred both horizontally (from fish to fish) and vertically (from female to juvenile via the egg).</p> <p>They include species that cause many of the most serious and untreatable diseases in finfish. The possibility that several viruses could be introduced with the transfer of Nile tilapia for aquaculture development is a serious concern.</p>
Bacteria	Direct	Yes	<p>Species that infect tilapia and other finfish are ubiquitous in aquatic environments.</p> <p>These are often opportunistic pathogens that cause disease in aquaculture facilities when fish are grown under stressful or suboptimal conditions.</p>
Protista			
Ciliata	Direct	Yes	<p>These are mostly ectoparasitic and often ubiquitous. Some species are host specific. Transfer occurs between hosts through direct contact or free-swimming stages. They are often present in low numbers in aquaculture facilities where they can cause disease when fish are raised under stressful conditions.</p>
Flagellata	Mostly Direct	Yes [some]	<p>Flagellata are mostly ectoparasitic and often ubiquitous. Some are host specific. Transfer occurs between hosts through direct contact or free swimming. They can cause serious diseases in finfish, particularly in aquaculture, and are often present in low numbers in aquaculture facilities.</p> <p>Certain flagellates having indirect life cycles (e.g. <i>Trypanosoma</i> and some <i>Cryptobia</i>) infect the blood of fish and are transmitted by the feeding activities of a leech vector.</p>
Amoebida	Direct	Yes	<p>A few genera (e.g. <i>Entamoeba</i>, <i>Schizamoeba</i>) are considered endocommensals that, under certain conditions, can become true parasites of fish (Lom and Dyková 1992). They are generally free-living forms that are opportunistic in fish.</p>
Sporozoa	Direct or Indirect	Yes [Some]	<p>Some species are reported to be transmitted via a leech or hemophagus crustacean vector (i.e. <i>Babesiosoma mariae</i>, <i>Haemogregarina</i> sp.) (Lom and Dyková 1992).</p>

Taxonomic group	Type of life cycle	Considered in hazard identification?	Remarks
<i>Myxosporidia</i>	Indirect	No	<p>These are common parasites of freshwater and marine fish that are often highly host specific.</p> <p>Some genera produce macroscopic cysts in the musculature, gills and/or viscera (e.g. <i>Henneguya</i>, <i>Myxobolus</i>).</p> <p>Life cycles require an obligate alternate host (a tubificid worm) in which the actinomyxin stage develops.</p> <p>Some species are highly pathogenic to fish, but infections can be prevented in hatchery situations by elimination of tubificids and treatment of incoming water to kill actinomyxins.</p>
Fungi			
Septate fungi	Direct	Yes [1]	<p>Most species are ubiquitous and opportunistic pathogens of fish. One species (<i>Aphanomyces invadans</i>), the cause of epizootic ulcerative syndrome (EUS), is a serious pathogen of freshwater and brackish-water fish.</p>
Microspora	Direct	Yes	<p>Fish microspora are transmitted directly perorally, and the existence of a paratenic or intermediate host has not been shown (Lom and Dyková 1992).</p> <p>Species are often highly host specific.</p>
Monogenea (monogeneans)	Direct	Yes	<p>Almost all species are ectoparasitic on the skin and/or gills. One genus occurring in tilapia species (<i>Enterogyrus</i>) is parasitic in the stomach.</p> <p>Most species lay eggs that hatch to ciliated larvae; however, some genera (e.g. <i>Gyrodactylus</i>) are viviparous.</p> <p>Monogeneans often are highly host specific, and there are many species that occur only on tilapia.</p>
Digenea (digenetic trematodes)	Indirect	No	<p>Digeneans infect Nile tilapia as either adults, occurring most often in the digestive tract, or as larvae (metacercariae) encysted in various parts of the body (i.e. the viscera, gills, musculature, eyes, etc.). One species occurring in tilapia (<i>Transversotrema cichlidarum</i>) is parasitic on the skin.</p> <p>Life cycles of digeneans infecting fish typically involve three hosts: a first intermediate molluscan host (usually a snail), a second intermediate host (usually a crustacean or a fish) and a definitive (or final) host (a fish or a piscivorous bird or mammal).</p>

Taxonomic group	Type of life cycle	Considered in hazard identification?	Remarks
<i>Cestoidea</i> (tapeworms)	Indirect	No	Life cycles involve a crustacean as the first intermediate host. Fish may serve as final hosts for some genera, or as second intermediate hosts, harboring the plerocercoid stage, which develops to adult in a piscivore.
<i>Acanthocephala</i> (thorny headed worms)	Indirect	No	These typically occur as adults in the intestine of fish. They require a single intermediate host (a crustacean) in which juveniles develop that infect the fish when the host is ingested.
<i>Nematoda</i> (roundworms)	Indirect	No	<p>There are many species of Nematoda. They occur as either adults, typically in the digestive tract of the fish host, or as larvae, encapsulated on the viscera or in the musculature. Larval stages occurring in the musculature of fish may be unsightly, causing loss of market value and/or may cause disease in humans if ingested through consumption of raw fish.</p> <p>Life cycles involve a first intermediate host (a crustacean), a second intermediate or transport host (another crustacean or a fish) and a final host (a fish or a piscivorous bird or mammal).</p> <p>Species often show some degree of host specificity.</p>
<i>Crustacea</i> (Branchiura, Copepoda and Isopoda)	Direct	Yes	<p>Branchiurans and parasitic copepods typically infect the skin and/or gills of fish, while isopods are often found in the buccal cavity.</p> <p>Adults produce eggs that hatch into free-swimming larvae that seek out and infect new fish hosts. Adults of most species are macroscopic and easily noticed in aquaculture situations.</p>
<i>Pentastomida</i> (tongue worms)	Indirect	No	Infections cannot be transmitted directly from fish to fish. Reptiles serve as final hosts of pentastome species that infect fish as nymphs.
<i>Hirudinea</i> (leeches)	Direct	Yes	Adults are macroscopic and thus, their presence is easily detected in aquaculture situations. Adults leave the fish host and produce cocoons on bottom substrates that contain eggs. These then hatch directly into juveniles that infect new piscine hosts.
<i>Mollusca</i>	Direct	Yes	Only the glochidial stage of unionid clams is a temporary parasite of the gills of freshwater fish.

Table 2. Summary of the life-cycle patterns of pathogens and parasites of Nile tilapia.

- Low: Establishment of disease would have mild biological consequences and would normally be amenable to control or eradication. Such diseases may harm economic performance at an industry level for a short period and/or may cause some minor environmental effects, which would not be serious or irreversible.
- Negligible: Establishment of disease would have no significant biological consequences and would require no control or eradication. Such diseases would not affect economic performance at an industry level and would cause negligible environmental effects.

4.7. Estimating likelihood of entry and exposure and estimating total risk

Estimates of the likelihood of an event occurring are combined using the matrix given in Table 3. This matrix is used to calculate the Likelihood of Entry (L_{Ent}), the Likelihood of Exposure (L_{Exp}) and the Likelihood of Entry and Exposure ($L_{Ent} \times L_{Exp}$). The total risk a hazard poses is estimated using the matrix given in Table 4.

		Estimated Likelihood of Event 1				
		Negligible	Very Low	Low	Moderate	High
Estimated Likelihood of Event 2	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible
	Very Low	Negligible	Negligible	Very Low	Very Low	Very Low
	Low	Negligible	Very Low	Very Low	Low	Low
	Moderate	Negligible	Very Low	Low	Low	Moderate
	High	Negligible	Very Low	Low	Moderate	High

Table 3. Matrix for combining risk likelihoods.

		Estimated Consequence of Entry and Exposure				
		Negligible	Low	Moderate	High	Catastrophic
Estimated Likelihood of Entry and Exposure	Negligible	Negligible Risk	Negligible Risk	Negligible Risk	Negligible Risk	Negligible Risk
	Very Low	Negligible Risk	Negligible Risk	Very Low Risk	Low Risk	Moderate Risk
	Low	Negligible Risk	Very Low Risk	Low Risk	Moderate Risk	High Risk
	Moderate	Negligible Risk	Low Risk	Moderate Risk	High Risk	Extreme Risk
	High	Negligible Risk	Low Risk	Moderate Risk	High Risk	Extreme Risk

Table 4. Matrix for estimating the total risk posed by a hazard.

5. Diseases of Nile tilapia

5.1. Transboundary spread of tilapia diseases

Nile tilapia are host to a wide range of pathogens and parasites (Table 5). Regarding aquaculture development, the most important are viruses, many of which the OIE (2019a) has listed as notifiable diseases having the potential to cause major economic, environmental and/or social impacts.

Many TAADs have been spread domestically, regionally and internationally, through both legal and illegal channels, by the ill-considered introduction and transfer of live aquatic animals to fuel the rapid expansion of aquaculture, often with disastrous consequences. The rapid emergence and spread of serious diseases of Nile tilapia on a regional and global basis is primarily the result of poor industry practices, including (a) the careless transboundary movement of broodstock and fry of unknown or poorly known health status, and (b) the common practice of siting both quarantine and production facilities near natural waterbodies where the likelihood of transferring pathogens between cultured stocks and wild cichlids is greatly increased. Also, the industry has often failed to recognize newly emerging diseases. This has resulted in the global spread of serious pathogens, such as TiLV, before reliable diagnostic methods have been developed to test for them.

5.2. Pathogens and parasites of *Oreochromis niloticus*⁶

As *O. niloticus* is by far the most widely cultured tilapia species globally, its pathogens and parasites are the most extensively documented among all cichlid species. Table 6 lists 69 taxa (species or species groups) of pathogens and parasites known to infect Nile tilapia and of potential concern to this risk analysis.

Of the diseases listed by the OIE, none has been reported from *O. niloticus*. Two have been reported from other tilapia species: (1) EUS from *O. aureus* and *O. mossambicus* from Zimbabwe and from *Tilapia* sp. from Botswana and (2) infectious pancreatic necrosis virus (IPNV) from *Tilapia* sp. from Kenya (Cefas 2020).

5.3. Pathogens and parasites of tilapia in Nigeria

A detailed knowledge of a country's aquatic viruses, bacteria, fungi and parasites, their pathogenicities and host and geographic distributions is fundamental to achieving healthy stocks in aquaculture production, protecting wild fish populations, and conducting pathogen risk analyses. Unfortunately, such knowledge remains very limited for Nigeria. The International Database on Aquatic Animal Diseases (Cefas 2020) contains no reports for Nigeria, either official or unofficial, of those aquatic animal diseases listed by the OIE. Sections 5.3.1 to 5.3.3 briefly discuss the state of knowledge, based on a comprehensive yet incomplete examination of the relevant literature.

5.3.1. Viral diseases

There have been no detailed studies of the viral pathogens of Nigerian fish. The only viral disease reported from Nigeria is lymphocystis (Okaeme et al. 1988).

5.3.2. Bacterial diseases

Bacterial infections are reported to be a major cause of losses to Nigerian aquaculture (Okaeme and Ibiwoye 1989; Ikpi and Offem 2010; Oladele et al. 2010 and 2015). However, there have been less than 50 studies of the bacteria causing disease in Nigerian fish culture (Annex 1). A few examples follow: In their review of problems in the culture of tilapia and *Clarias* in Nigerian freshwaters, Okaeme and Ibiwoye (1989) listed eight taxa (*Pseudomonas putrifacium*, *Pseudomonas* sp., *Aeromonas hydrophila*, *Myxobacteria* sp., *Myxococcus* sp., *Staphylococcus* sp., *Proteus mirabilis* and *Enterobacter aerogenes*) as common pathogens of tilapia. Shinkafi and Ukwaja (2010) identified the bacteria associated with fresh Nile tilapia purchased at a market in Sokoto, reporting six Gram-positive species (*Bacillus megaterium*, *B. pumilus*, *B. alvei*, *B. licheniformis*, *Staphylococcus saprophyticus*, *Listeria monocytogenes*), and three Gram-negative bacteria (*Serratia mercerscens*, *Providentia stuartii*, *Salmonella* spp.). However, none of these taxa appear to have been associated

with disease outbreaks in aquaculture. Ashiru et al. (2011) isolated *Aeromonas* spp. (*A. caviae*, *A. hydrophila*, *A. sobria*) from the body surface and intestine of *O. niloticus* and/or *Clarias batrachus* purchased from a market in Lagos and reported on their resistance to several antibiotics. Oladele et al. (2012) implicated hemolytic strains of *Staphylococcus aureus* in outbreaks of jaundice syndrome in clariid catfish, while Oladele et al. (2011) associated *A. sobria* with arborescent organ necrosis syndrome. A major survey of bacteria present in cultured fish was conducted by Oladele et al. (2015). They isolated the following taxa from diseased fish examined from commercial catfish farms (hatcheries and grow-out ponds) in southwestern Nigeria: *Staphylococcus aureus*, *Staphylococcus* spp., *Enterococcus* spp., *Luteococcus sanguinis*, *Klebsiella oxytoca*, *Aeromonas hydrophila*, *A. sobria*, *Streptococcus* spp., *Bacillus cereus*, *Vibrio alginolyticus*, *V. parahemolyticus*, *Bacillus* spp. and *Corynebacterium accolence*.

5.3.3. Fungi

Filamentous fungi such as *Saprolegnia* spp. are frequent opportunistic pathogens of freshwater fish. Of the nearly 400 publications dealing with pathogens of Nigerian freshwater fish, only four studies deal with fungal infections (Annex 1). For example, Ogbonna and Alabi (1991) recorded a diversity (24 species belonging to 6 genera) of aquatic phycomycete fungi from infected fish in a freshwater pond.

5.3.4. Parasites

Because of the vast literature dealing with Nigerian fish parasites (some 350 publications were encountered, see Annex 1), the inaccessibility of many of these publications and the limited time allowed for this study, this section is restricted to a consideration of the parasites of the cichlid species reported from Nigeria.

Nigerian workers have published several rather incomplete reviews dealing with the parasites of Nigerian freshwater fish. Adebambo (2020), for example, listed only 55 publications as containing original records. In a review of the parasites of catfish and tilapia in the wild and homestead ponds in Nigeria, Flourizel et al. (2019) cited only a single publication related to tilapia parasites. Okaeme et al.

(1988 and 2001) briefly reviewed the parasites reported from tilapia of the Lake Kainji area.

More general publications dealing with the parasites of freshwater fish of Africa include the following:

- the guides of Paperna (1996) and Scholz et al. (2020)
- the checklist of parasites of freshwater fish of Southern Africa by Van As and Basson (1984)
- the listing of pathogens and parasites of East African freshwater fish by Akoll and Mwanja (2012)
- the checklist of helminths of African freshwater fish by Khalil (1971), updated by Khalil and Polling (1997).

To date, there has not been a comprehensive, critical review or detailed checklist of the parasites reported from Nigerian fish.

Research on the fish parasites of Nigeria appears to have been hampered by a lack of specialized taxonomic expertise as well as limited access to essential literature. As a result, many studies have not been able to identify parasite taxa to species, while other studies appear to include incorrect identifications. Unfortunately, many of the papers published by Nigerian workers have appeared in online journals that have often provided inadequate peer review or skilled editing support. In only few cases have authors sent specimens to international experts to confirm identifications or deposited voucher specimens in a recognized museum so that their identifications could be verified later. Almost all parasitological attention has focused on a few species of catfish (genera *Clarias*, *Chrysichthys* and *Synodontis*) and tilapia (genera *Oreochromis*, *Coptodon*, *Sarotherodon*, *Hemichromis*, *Chromidotilapia* and *Pelmatolapia*). FishBase (Froese and Pauly 2019) provides an incomplete listing of the diverse fish fauna of Nigeria, with a total of 803 species, of which 478 are marine and 334 are freshwater species. Of these, 21 belong to the family Cichlidae (the tilapia species) and, of these, records of pathogens or parasites appear in the Nigerian literature for only 11 (Table 5). The end result is that despite considerable efforts, the parasites of Nigerian fish remain poorly known.

Table 5 lists the parasites reported from Nigerian cichlids. Although this list undoubtedly includes a number of misidentifications, it does provide an indication of the diverse parasite fauna present in

Nigerian cichlids. This list includes some 78 genera of parasites, as follows: 14 Protista, 1 Myxosporea, 4 Monogenea, 11 Digenea, 13 Cestoda, 20 Nematoda, 5 Acanthocephala, 5 Crustacea and 5 Hirudinea.

Taxon	Host species	References
Protista		
"Amoeba"	<i>Oreochromis niloticus</i>	Okaeme et al. 1987
<i>Babeiosoma</i> sp.	<i>O. niloticus</i>	Biu et al. 2014
<i>Chilodonella</i> sp.	<i>O. niloticus</i>	Nyaku et al. 2007; Ashade et al. 2010; Osoh et al. 2017
<i>Cryptobia</i> sp.	<i>Coptodon zillii</i> <i>O. niloticus</i> <i>Satherodon galilaeus</i>	Osoh et al. 2017
<i>Eimeria</i> sp.	<i>C. zillii</i> <i>O. niloticus</i> <i>S. galilaeus</i>	Okaeme et al. 1987
<i>Entamoeba</i> sp.	<i>S. melanotheron</i>	Akinsanya et al. 2018a
<i>Epistylis</i> sp.	<i>O. niloticus</i>	Ashade et al. 2010
<i>Haemogregarina</i> sp.	<i>O. niloticus</i>	Biu et al. 2014
<i>Hexamita</i> sp.	<i>O. niloticus</i> <i>S. galilaeus</i> <i>S. melanotheron</i>	Okaeme et al. 1987; Osoh et al. 2017; Akinsanya et al. 2018a
<i>Ichthyobodo necatrix</i>	<i>C. zillii</i> <i>O. niloticus</i> <i>S. melanotheron</i>	Omoniyi and Ojelade 2017
<i>Ichthyophthirius multifiliis</i>	<i>C. zillii</i> (syn: <i>Tilapia melanopleura</i>) <i>O. niloticus</i> <i>S. galilaeus</i>	Okaeme et al. 1987; Nyaku et al. 2007; Ashade et al. 2010; Abidemi-Iromini and Eze 2011; Bello-Olusoji et al. n.d.; Osoh et al. 2017; Enyidi and Uwanna 2019
<i>Piscinoodinium</i> sp.	<i>O. niloticus</i>	Nyaku et al. 2007
<i>Tetrahymena</i> sp.	<i>O. niloticus</i>	Nyaku et al. 2007
<i>Trichodina acuta</i>	<i>C. zillii</i> <i>O. niloticus</i>	Bello-Olusoji et al. n.d.; Abidemi-Iromini and Eze 2011
<i>Trichodina heterodentata</i>	<i>O. niloticus</i>	Enyidi and Uwanna 2019
<i>Trypanosoma tincae</i>	<i>C. zillii</i> <i>O. niloticus</i>	Bello-Olusoji et al. n.d.
<i>Trypanosoma toddi</i>	<i>Hemichromis</i> sp.	Abolarin 1970

Taxon	Host species	References
Myxosporea		
<i>Myxobolus agolus</i>	<i>C. guineensis</i> <i>O. niloticus</i> <i>S. galilaeus</i> and hybrids	Obiekezie and Okaeme 1990
<i>Myxobolus brachyspora</i>	<i>C. guineensis</i> <i>O. niloticus</i> <i>S. galilaeus</i> and hybrids	Obiekezie and Okaeme 1990
<i>Myxobolus cyprini</i>	<i>C. zillii</i> <i>O. niloticus</i>	Bello-Olusoji et al. n.d.
<i>Myxobolus equitoralis</i>	<i>C. guineensis</i> <i>O. niloticus</i> <i>S. galilaeus</i>	Obiekezie and Okaeme 1990
<i>Myxobolus galilaeus</i>	<i>O. niloticus</i> <i>S. galilaeus</i> and hybrids	Obiekezie and Okaeme 1990
<i>Myxobolus homeospora</i>	<i>O. niloticus</i>	Obiekezie and Okaeme 1990
<i>Myxobolus israelensis</i>	<i>C. guineensis</i> <i>O. niloticus</i> <i>S. galilaeus</i> and hybrids	Okaeme et al. 1987; Obiekezie and Okaeme 1990
<i>Myxobolus kainjiae</i>	<i>O. niloticus</i> <i>S. galilaeus</i>	Obiekezie and Okaeme 1990
<i>Myxobolus sarigi</i>	<i>O. niloticus</i> <i>S. galilaeus</i> and hybrids	Okaeme et al. 1987; Obiekezie and Okaeme 1990
<i>Myxobolus tilapiae</i>	<i>C. zillii</i> <i>O. niloticus</i> <i>S. galilaeus</i>	Abolarin 1974; Obiekezie and Okaeme 1990
Monogenea		
<i>Dactylogyrus extensus</i>	<i>O. niloticus</i>	Enyidi and Uwanna 2019
<i>Enterogyrus cichlidarum</i>	<i>O. niloticus</i>	Musa et al. 2007
<i>Gyrodactylus malalai</i>	<i>O. niloticus</i>	Adeshina et al. 2021
<i>Gyrodactylus vastator</i> [probable misidentification]	<i>C. zillii</i> <i>O. niloticus</i>	Bello-Olusoji et al. n.d.
<i>Macrogyrodactylus</i> sp.	<i>O. niloticus</i>	Ashade et al. 2010
Digenea		
<i>Allocreadium ghanensis</i>	<i>C. zillii</i> <i>S. galilaeus</i> <i>S. melanotheron</i> "cichlids"	Morenikeji and Adepeju 2009; Simon-Oke and Morenikeji 2015; Simon-Oke 2017

Taxon	Host species	References
<i>Alloglossidium corti</i>	<i>C. zillii</i> <i>S. galilaeus</i> <i>S. melanotheron</i> "cichlids"	Morenikeji and Adepeju 2009; Hassan et al. 2013; Simon-Oke and Morenikeji 2015; Simon-Oke 2017
<i>Aranthocephalus</i> sp. metacercaria [lapsus?]	<i>O. niloticus</i> <i>S. galilaeus</i>	Okaeme et al. 1987
<i>Clinostomum complanatum</i> metacercaria	<i>C. guineensis</i> <i>C. zillii</i> <i>O. niloticus</i> <i>S. melanotheron</i>	Echi et al. 2009a, 2009b, 2012, 2014; Amaechi 2015
<i>Clinostomum marginatum</i> metacercaria	<i>O. niloticus</i>	Ashade et al. 2010
<i>Clinostomum tilapiae</i> metacercaria	<i>Chromidotilapia guntheri</i> <i>Coptodon guineensis</i> <i>C. zillii</i> <i>Hemichromis elongatus</i> <i>H. fasciatus</i> <i>O. niloticus</i> <i>S. galilaeus</i> <i>S. melanotheron</i> <i>Pelmatolapia mariae</i> (syn. <i>T. mariae</i>) "cichlids" "tilapias"	Okaeme et al. 1987; Okaeme and Ibiwoye 1989; Musa et al. 2007; Adeyemo and Agbede 2008; Morenikeji and Adepeju 2009; Echi et al. 2009a, 2009b, 2012, 2014; Olurin et al. 2012; Hassan et al. 2013; Amaechi 2015; Ajala and Fawole 2015; Simon-Oke and Morenikeji 2015; Awharitoma and Ehigiator 2017; Simon-Oke 2017; Olugbotemi and Morenikeji 2018; Osimen and Anagha 2020
<i>Crepidostomum metoecus</i> [probable misidentification]	<i>C. zillii</i>	Yakubu et al. 2002
<i>Diplostomulum tregenna</i> metacercaria	<i>C. zillii</i>	Yakubu et al. 2002; Goselle et al. 2008
<i>Euclinostomium heterostomum</i> metacercaria	<i>C. guineensis</i> <i>C. zillii</i> <i>O. niloticus</i> <i>S. galilaeus</i> <i>S. melanotheron</i> "cichlids"	Okaeme et al. 1987; Ukpai 2001; Morenikeji and Adepeju 2009; Echi et al., 2009a, 2009b, 2012, 2014; Ohaeri 2012; Hassan et al. 2013; Saliu et al. 2014; Amaechi 2015; Simon-Oke and Morenikeji 2015; Simon-Oke 2017
<i>Heterophyes</i> sp. metacercaria	<i>O. niloticus</i>	Ashade et al. 2010
<i>Neascus</i> sp. metacercaria	<i>C. zillii</i> <i>O. niloticus</i> <i>S. galilaeus</i> <i>S. melanotheron</i> "cichlids" "tilapias"	Okaeme et al. 1987; Okaeme and Ibiwoye 1989; Morenikeji and Adepeju 2009; Hassan et al. 2013; Simon-Oke and Morenikeji 2015; Simon-Oke 2017; Abba et al. 2018a, 2018b
<i>Phagicola longa</i>	<i>C. zillii</i> <i>H. bimaculatus</i> <i>H. fasciatus</i> <i>O. niloticus</i> <i>S. galilaeus</i> <i>S. melanotheron</i> "cichlids"	Morenikeji and Adepeju 2009; Simon-Oke and Morenikeji 2015; Hassan et al. 2013; Simon-Oke 2017

Taxon	Host species	References
<i>Sphaerostoma bramae</i> [probable misidentification]	<i>C. zillii</i>	Yakubu et al. 2002
Cestoda		
<i>Anomotaenia</i> sp. larva	<i>H. fasciatus</i> <i>O. niloticus</i>	Aderounmu and Aeniya 1972
<i>Biacetabulum</i> [?] <i>appendiculatum</i>	<i>Ch. guntheri</i>	Osimen and Anagha 2020
<i>Bothriocephalus aegyptiacus</i>	<i>O. niloticus</i>	Abba et al. 2018a, 2018b
<i>Caryophyllaeides</i> sp.	<i>C. zillii</i>	Omoniyi and Ojelade 2017
<i>Caryophyllaeus</i> sp.	<i>C. zillii</i> "tilapia"	Ukpai 2001; Biu and Nkechi 2013
<i>Diphyllobothrium latum</i> plerocercoid [probable misidentification]	<i>O. niloticus</i>	Edeh and Solomon 2016; Awosolu et al. 2018
<i>Eubothrium crassum</i> [probable misidentification]	<i>C. zillii</i>	Yakubu et al. 2002
<i>Hymenolepis nana</i> [probable misidentification]	<i>C. zillii</i>	Iyabo and Ijeoma 2017
<i>Monobothrium</i> sp.	<i>C. zillii</i>	Alade et al. 2015
<i>Paradilepis</i> sp. larvae	<i>C. guineensis</i> <i>O. niloticus</i>	Ezeri 2002; Joseph et al. 2020
<i>Proteocephalus</i> sp.	<i>C. guineensis</i> <i>C. zillii</i> <i>O. niloticus</i>	Saliu et al. 2014; Onoja-Abutu et al. 2021
<i>Polyonchobothrium clarias</i>	<i>C. zillii</i>	Alade et al. 2015; Mgbemena et al. 2020
<i>Wenyonia minuta</i>	<i>S. melanotheron</i>	Akinsanya et al. 2018b
Nematoda		
<i>Ascaris</i> sp. [probable misidentification]	<i>C. zillii</i> <i>O. niloticus</i> <i>S. galilaeus</i>	Osoh et al. 2017
<i>Camallanus polypteri</i>	<i>C. zillii</i> <i>O. niloticus</i>	Ejere et al. 2014; Enyidi and Uwanna 2019
<i>Capillaria cichlasomae</i>	<i>C. zillii</i>	Ejere et al. 2014
<i>Contraecum osculatum</i>	<i>Ch. guntheri</i> <i>C. zillii</i> <i>O. niloticus</i>	Osimen and Anagha 2020
<i>Cucullanus barbi</i>	<i>Ch. guntheri</i>	Edema et al. 2008
<i>Cucullanus baylisi</i>	<i>C. zillii</i> <i>O. niloticus</i> <i>S. galilaeus</i>	Ibiwoye et al. 2006

Taxon	Host species	References
<i>Cucullanus sheilansensis</i>	<i>C. zillii</i>	Akinsanya 2016
<i>Dichelyne</i> sp.	<i>Ch. guntheri</i>	Awharitoma and Ehigiator 2017
<i>Eustrongyldes</i> sp. larvae	<i>C. zillii</i> <i>O. niloticus</i>	Ashade et al. 2010; Sikoki et al. 2013; Sani et al. 2019; Mgbemena et al. 2020
<i>Gnathostoma spinigerum</i> larvae	<i>O. niloticus</i>	Awosolu et al. 2018
<i>Goezia sigalasi</i>	<i>O. niloticus</i>	Sikoki et al. 2013
<i>Philonema</i> sp.	<i>O. niloticus</i>	Sani et al. 2019
<i>Paracamallanus cyathopharynx</i>	<i>S. galilaeus</i> "tilapia"	Ukpai 2001; Ajala and Fawole 2015
<i>Procamallanus laevionchus</i>	<i>C. guineensis</i> <i>C. zillii</i> <i>O. niloticus</i> <i>S. galilaeus</i>	Opara and Okon 2002; Ibiwoye et al. 2006; Okogwu et al. 2011; Alade et al. 2015; Ajala and Fawole 2015; Awharitoma and Ehigiator 2017; Abba et al. 2018a, 2018b; Osimen and Anagha 2020
<i>Raphidascaroides</i> sp.	<i>C. zillii</i>	Akinsanya et al. 2018b
<i>Rhabdochona congolensis</i>	<i>C. zillii</i> <i>O. niloticus</i>	Onoja-Abutu et al. 2021
<i>Serradacnitis serrata</i>	<i>O. niloticus</i>	Domo and Ester 2015
<i>Spinitectus guntheri</i>	<i>C. zillii</i> <i>O. niloticus</i>	Onoja-Abutu et al. 2021
<i>Spirocamallanus spiralis</i>	<i>H. elongatus</i>	Awharitoma and Ehigiator 2017
<i>Spironoura petrei</i>	<i>O. niloticus</i> <i>S. galilaeus</i>	Ibiwoye et al. 2006
<i>Trichiuris</i> sp. [probable misidentification]	<i>O. niloticus</i>	Ashade et al. 2010
<i>Trichostrongylus</i> sp. [probable misidentification]	<i>C. zillii</i>	Iyabo and Ijeoma 2017
Acanthocephala		
<i>Acanthogyrus</i> (<i>Acanthosentis</i>) <i>tilapiae</i>	<i>Ch. guntheri</i> <i>C. zillii</i> <i>H. bimaculatus</i> <i>H. elongatus</i> <i>H. fasciatus</i> <i>S. galilaeus</i> <i>S. melanotheron</i> <i>O. aureus</i> (syn.: <i>T. aurea</i>) <i>O. niloticus</i> <i>P. mariae</i> "cichlids" "tilapias"	Shotter 1974; Hyslop 1988; Okaeme and Ibiwoye 1989; Morenikeji and Adepeju 2009; Matuoke et al. 2011; Hassan et al. 2013; Ajala and Fawole 2015; Simon-Oke and Morenikeji 2015; Awharitoma and Ehigiator 2017; Simon-Oke 2017; Ito 2017; Atalabi et al. 2018
<i>Neoechinorhynchus rutili</i>	<i>C. zillii</i> <i>O. niloticus</i> <i>S. galilaeus</i>	Olurin et al. 2012; Alade et al. 2015; Ajala and Fawole 2015; Abba et al. 2018a, 2018b

Taxon	Host species	References
<i>Octospiniferoides</i> sp.	cichlids	Nmor et al. 2004
<i>Pomphorhynchus</i> sp.	<i>O. niloticus</i>	Sani et al. 2019
<i>Rhadinorhynchus horridus</i>	<i>H. elongatus</i>	Awharitoma and Ehigiator 2017
Crustacea		
<i>Argulus africana</i>	<i>O. niloticus</i> "all tilapias"	Okaeme et al. 1987; Okaeme and Ibiwoye 1989
<i>Ergasilus latus</i>	<i>O. niloticus</i> <i>S. galilaeus</i> <i>T. zillii</i>	Schlebusch 2014
<i>Lamproglena monodi</i>	<i>O. niloticus</i> "all tilapias"	Okaeme et al. 1987; Okaeme and Ibiwoye 1989
<i>Lernaea cyprinacea</i>	<i>O. niloticus</i>	Enyidi and Uwanna 2019
<i>Lernaeocera branchialis</i>	<i>O. niloticus</i>	Sikoki et al. 2013
Hirudinea		
<i>Batrachobdelloides tricarinata</i>	<i>C. guineensis</i> <i>C. zillii</i> <i>S. melanotheron</i>	Echi 2016
<i>Cystobranchnus</i> sp. [possible food item]	<i>O. niloticus</i>	Sani et al. 2019
<i>Haementeria</i> sp. [possible food item]	<i>O. niloticus</i>	Sani et al. 2019
<i>Illinobdella</i> sp. [possible food item]	<i>O. niloticus</i>	Sani et al. 2019
<i>Piscicola geometra</i>	<i>C. zillii</i> <i>O. niloticus</i>	Opara 2002; Ejere et al. 2014

Note: where records for both named species and identifications to genus only exist, just the named species is listed.

Table 5. Parasites reported from the cichlid fish of Nigeria.

6. Health status of GIFT

The parental stock (G17) of the GIFT swim-up fry to be transferred to Nigeria will originate from WorldFish's GIFT broodstock facility (a secure holding/breeding facility) in Malaysia. The stock is derived from WorldFish's original core GIFT selective breeding stock maintained at Jitra in Malaysia. Following the first reports of TiLV in Malaysia in 2016 and considering the risks to WorldFish's core GIFT selective breeding program at Jitra, a backup selective breeding program was established on the premises of WorldFish's headquarters in Penang, Malaysia. In May 2017, a second transfer was made, with several families from G16 moved from Jitra to Penang.

Both transfers to the Penang facility took place well before the reported outbreak of TiLV that occurred in a GIFT stock contained in a single pond at the Jitra facility in February 2018. In November 2020, the Jitra facility was closed, and the Penang facility was transformed into WorldFish's GIFT broodstock facility, where the GIFT breeding population is now maintained.

To confirm their health status and freedom from TiLV, screening of backup stocks belonging to G14, G15 and G16 was conducted. Following this, detailed planning and mating designs were done to use these backup stocks to produce the desired number of G17 families. A rigorous health screening was adopted throughout the process. Fertilized eggs, swim-up fry and fingerlings (at tagging) of all G17 families were screened for TiLV using the most appropriate diagnostic methodology (i.e. the TaqMan qPCR (realtime polymerase chain reaction (PCR)) method. In addition to G17, all parents used (coming from G14, G15 and G16) were sacrificed post-breeding to check for freedom from TiLV. This entire process, conducted between January 2019 and July 2020, involved testing some 4774 fish samples. The results confirmed that the Penang broodstock is free from TiLV and that the G17 families now being raised to produce G17-F1 are free from TiLV. Regular and rigorous screening for TiLV started in October 2019 and all laboratory records are available.

As part of WorldFish's SOPs, broodstock are screened bi-annually for a list of pathogens (up to

12) by sampling up to 60 fish. Additionally, general health screening (based on wet smears and some histology) has been conducted on the fish held in the Penang facility since its establishment. Although no laboratory records have been kept, there have been no mortalities or TiLV outbreaks in this facility since its establishment in 2013.

The batch of swim-up fry to be transferred to Nigeria will originate from the health-screened G17 population in Penang. Pathogen screening will be conducted on samples taken from batches of fish that are being reared for export. The list of pathogens should take into consideration the OIE's reportable disease list, the Malaysian national pathogen list, the WorldFish priority list, the recommendations of this risk analysis and any additional screening requested by the GON. This work will be linked to Malaysia Biosecurity as part of its work for issuing the health certificate that will accompany the GIFT fry being shipped to Nigeria.

The batch of GIFT swim-up fry to be transferred to Nigeria will be produced in a clean and regularly disinfected hatchery facility under artificial incubation procedure. Before packing for transfer, the fry will be surface disinfected using standard methodology and then bagged in clean water.

A summary of the pathogens for which screening of broodstock was conducted in October/ November 2020 is given in Table 6. As provided by WorldFish, the results were negative for the presence of all pathogens for which testing was conducted. Another comprehensive health assessment will be conducted for the same batch of fish in March 2021, with the results expected around the end of April 2021.

It should be noted that broodstock held in or fry produced by WorldFish's GIFT broodstock facility should not be referred to as "SPF" or "high health." These are terms specific to certain stocks of penaeid shrimp that meet rigorous criteria with regard to their pathogen status. The definitions of these terms were formalized by the United States Marine Shrimp Farming Program in the 1980s and have recently been reviewed by Alday-Sanz et al. (2020). The SPF concept (in a slightly different form)

has also been applied in the salmonid culture industry, through the use of SPF eggs to prevent the movement of certain pathogens between facilities, and a stock of zebrafish (*Danio rerio*) that is SPF for a single pathogen is now available from Oregon State University (Kent et al. 2011). However, all stocks of aquatic animals, whether SPF or not, may still carry some pathogens.

In the development of SPF fishstocks, egg-laying fish provide an advantage over live-bearing fish in that pathogen exposure to the next generation can be reduced by separating eggs from their parents and disinfecting them before introducing

them into another facility. The introduction of new stocks via chlorine surface-disinfected eggs allows for the possibility of applying the same principles and methods routinely used in salmonid aquaculture for establishing SPF stocks to other fish species, such as screening broodstock and sex products and rearing fry in an environment completely separate from potentially infected fish, including broodstock (Kent et al. 2011). However, in the current transfer, as Nile tilapia are mouth brooders, and eggs will not be immediately separated from parent fish, there is a greatly increased opportunity for pathogens to be transferred directly from parent to offspring.

Population	No. of samples	Type of samples	Sampling date	Analysis date	Pathogens screened ¹	Result
Penang G17	60	Pooled liver, spleen and kidney	15–6/10/2020	2–8/12/2020	Iridovirus	Negative
					ISKNV	Negative
					EUS	Negative
					SA	Negative
					SI	Negative
					KHV	Negative
					CEV	Negative
					EHN	Negative
					TiLV	Negative

¹ ISKNV = infectious spleen and kidney necrosis virus, EUS = epizootic ulcerative syndrome caused by *Aphanomyces invadans*, SA = *Streptococcus agalactiae*, SI = *S. iniae*, KHV = koi herpesvirus, CEV = carp edema virus, EHN = epizootic hematopoietic necrosis virus, TiLV = tilapia lake virus.

Note: baseline health assessment for 9 pathogens (6 virus, 2 bacteria and 1 fungus) using conventional PCR.

Table 6. Results of baseline health screening of GIFT broodstock (G17) held in Penang.

7. Risk management measures

WorldFish has proposed the risk management measures discussed in sections 7.1 to 7.9, with some already in progress.

7.1. Use of fry derived from the GIFT breeding program broodstock

WorldFish proposes to biosecurely transfer an initial batch of 10,000 GIFT swim-fry from its GIFT broodstock facility in Malaysia to Nigeria in 2021. Fry will be produced using G17 GIFT as parents. Transferred fry will be kept in a designated land-based, secured quarantine facility in Nigeria's Ogun State, where they will be raised with regular health checks. G17-F3 progeny produced from G17-F2 fish resulting from the originally transferred stock (G17-F1) will be transferred to Delta State for breeding (non-sex reversed fry weighing 10 g) and for grow-out (sex reversed all male fry weighing 2 g). If this plan is not achieved for any reason, at any state of the process, all remaining fish will be destroyed, and all facilities will be cleaned and fallowed adequately.

7.2. Documented testing for pathogens of GIFT broodstock and fry

The GIFT strain has been housed at the Penang facility without addition of new broodstock since 2017 and has been subjected to a number of diagnostic tests since the initial establishment of the facility in 2013. A summary of the most recent diagnostic testing that has been performed on the GIFT stock is given in Table 6. In particular, extensive testing has been done to assure that the stock is free of TiLV (Section 6).

7.3. High-security quarantine of imported fry

Upon arrival in Nigeria, the swim-up fry will be housed in a biosecure, land-based quarantine facility to be constructed in Ogun State that will prevent their escape and that of subsequent generations. Quarantine facilities will meet minimum standards of construction and will follow SOPs appropriate to such high-containment facilities, as outlined in Section 4 of Arthur et al. (2007). Construction and operating standards will also minimize the possibility of diseases that may be present in the external environment gaining entry to the facility.

7.4. Monitoring and diagnostic testing of GIFT while in quarantine

Transferred stock and their progeny (G17-F1, F2, F3) kept in the biosecure quarantine facility in Ogun will be monitored for health on a daily basis. They will be tested for specified pathogens upon arrival and at 6-month intervals for 2 years before being released from the facility. Diagnostic testing will also be conducted should any unexplained mortalities occur. Any subpopulation infected with an untreatable exotic pathogen will be destroyed.

7.5. Releasing only G17–F3 GIFT to NBCs

The transferred GIFT will be used to produce G17–F2 and F3 progeny. All broodstock reared from the transferred fry used to develop the parent stock in Nigeria will be destroyed and disposed of in a sanitary manner (OIE 2019a) once they are no longer useful for breeding.

WorldFish will develop a list of pathogens of concern that will form the basis for subsequent diagnostic testing. Following successful testing, G17-F3 progeny will be released from the quarantine facility to be cultured in both land-based and water-based systems (ponds and cages) in Ogun and Delta states.

7.6. Diagnostic testing of Nigerian tilapia and catfish for some important pathogens

WorldFish has arranged to conduct diagnostic testing on selected target organs taken from catfish and tilapia currently being held in the facility that will be upgraded to become the GIFT high-biosecurity quarantine facility in Ogun. A certified diagnostics laboratory in Malaysia will test for a number of pathogens causing important diseases in tilapia culture. These will include four viral pathogens (TiLV, ISKNV, KHV and VNNV), the fungal pathogen (*Aphanomyces invadans*) that causes EUS, and seven bacterial pathogens (*Streptococcus agalactiae*, *S. iniae* and *S. dysagalactiae*, which are the causative agents of streptococcosis and *Aeromonas hydrophila*, *A. veronii*, *A. jandaei* and *A. schubertii*, which are causative agents of hemorrhagic septicemia).

The test results will reveal any pathogens currently present in the culture facility that may have the potential to infect the transferred GIFT fry.

7.7. Expert risk management panel

WorldFish has commissioned a panel of three experts to examine possible genetic, ecological and environmental, and pathogen risks and to recommend additional risk management measures to be incorporated into the strategic plan. This document is one of these studies.

7.8. Independent national scientific advisory team

WorldFish will set up an independent national advisory team consisting of representatives from key government agencies, the aquaculture sector and other stakeholders. Nigeria's competent authority, the Department of Fisheries and Aquaculture, will lead the group meetings and dialogue toward building consensus on the proposed risk management strategy.

7.9. Contingency planning

Considering all precautionary measures to be taken during the transfer, breeding, seed dissemination and grow-out of GIFT in Nigeria,

WorldFish considers that two emergency situations are possible. One is the identification of an important exotic pathogen (disease) in the transferred or subsequent populations (G17–F1, F2, F3 stocks) held in the quarantine/breeding facility. During the first 3 years, all stocks will be regularly tested for important pathogens. If infection by an important and untreatable exotic pathogen is found, WorldFish will destroy the infected subpopulation(s) to ensure the pathogen is not transferred to the outside environment. In addition, WorldFish will continue regular testing of other subpopulations for 2 years to ensure the facility is free from the pathogen of concern.

The second emergency situation is the escape of an exotic pathogen from the high-biosecurity facility and its establishment in the external aquatic environment through a breach of biosecurity. WorldFish does not foresee the accidental escape of GIFT from the broodstock holding and breeding facility, as all measures (infrastructure, engineering and management) will be taken and in place following introduction.

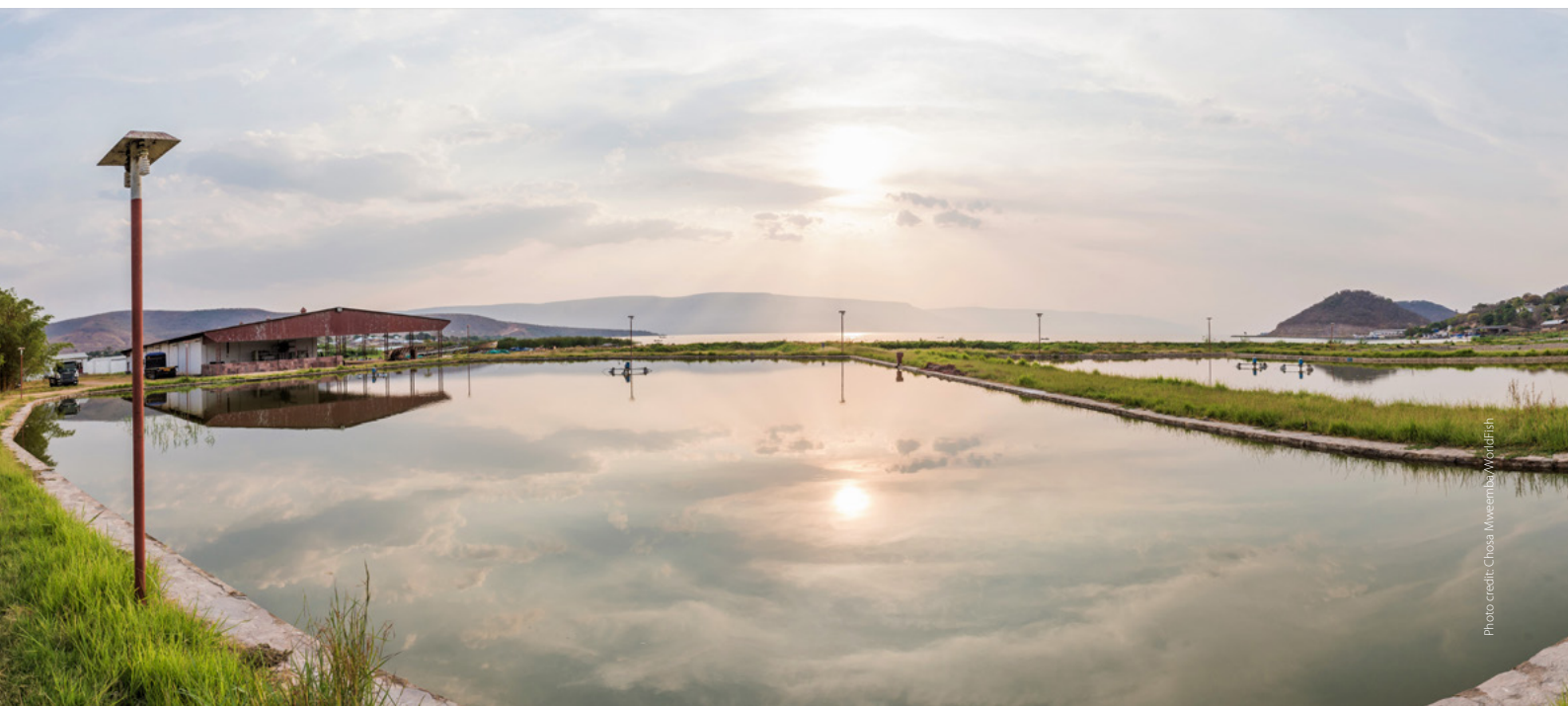


Photo credit: Chosa Mweemba, WorldFish

8. Hazard identification

8.1. Criteria to be considered a hazard

As outlined in Section 4.2, hazard identification will proceed in several steps. The first step will consider the pathogens and parasites reported from Nile tilapia and assess whether these “possible hazards” should be considered “potential hazards” to be given further screening. For a possible hazard to be given further consideration in the risk analysis, the following criteria must be fulfilled:

1. The pathogen must have been reported to infect Nile tilapia.
2. The agent must be an obligate pathogen (i.e. it is not a ubiquitous free-living organism that is capable of becoming an opportunistic pathogen of Nile tilapia under certain environmental or culture conditions).
3. The agent must cause significant disease outbreaks and associated losses in populations of Nile tilapia or, if not a significant pathogen of this species, it must cause serious disease outbreaks in populations of other species of tilapine fish.
4. It must be plausible that that the agent might be in the WorldFish’s GIFT breeding facility in Malaysia and in the shipment of GIFT fry that will be approved for importation to Nigeria.

The second step in hazard identification will take into account the various risk management measures in process or proposed by WorldFish. To be considered a hazard that requires additional risk management measures, the following criteria will be applied:

5. Should the pathogen be present in the imported GIFT fry, it must be plausible that the risk management measures proposed by WorldFish would be insufficient to prevent its transfer to Nigeria with infected fry (“entry”) and its escape or release from quarantine and infection of cultured and/or wild fish populations in Nigeria (“exposure”).

8.2. Results of the hazard identification: Preliminary screening

The results of this initial step are given in Table 7. Using criteria 1–4, a total of 69 “possible hazards” (pathogen species or species groups) have been considered, and 20 of these have been identified as “potential hazards” for further assessment. These include 7 viruses, 1 fungus, 8 protozoans, 2 monogeneans and 2 crustaceans.

8.3. Pathogens considered further

Preliminary hazard identification has identified 20 potential pathogens that require further assessment. These include 7 viruses, 1 fungus, 8 protozoans, 2 monogeneans and 2 crustaceans.

The 20 possible hazards that meet all of the requirements of preliminary screening are listed in Table 8. The risk management measures in progress or proposed by WorldFish are taken into consideration during the assessment of each potential hazard’s (a) “likelihood of entry” (i.e. the likelihood that the pathogen is present in GIFT fry and will be transferred from Malaysia to the border of Nigeria along with the exported shipment of fish), and (b) the “likelihood of exposure” (i.e. the likelihood that the hazard, having gained entry to Nigeria, will escape from the quarantine facility and become established in cultured or wild populations). This likelihood estimate (entry x exposure) is then combined with an estimate of consequence to obtain an estimate of total risk. It is important to note that should the estimated risk for a given hazard become “negligible” at any point in the risk assessment process, then the risk assessment for that individual hazard is stopped.

Pathogen	Infected Nile tilapia?	Infected Nile tilapia fry?	Causes significant disease?	Present in WorldFish facility?	Present in Nigeria?	Further consideration required?	Comments
VIRUSES							
Tilapia lake virus (TiLV)	Yes	Yes	Yes	Possible	Possible	Yes	<p>TiLV appears to be specific to tilapia. It has been reported from both cultured and wild tilapia, including Nile tilapia, hybrid tilapia (<i>O. niloticus</i> x <i>O. aureus</i>), red tilapia (<i>O. niloticus</i> x <i>O. mossambicus</i>), <i>O. aureus</i>, <i>Sarotherodon galilaeus</i>, <i>Coprodon zillii</i> and <i>Tristramellasisimonis intermedia</i> (FAO 2018). All species of tilapia are thus likely to be susceptible.</p> <p>Associated mortalities are reported as ranging from moderate (5%–20%) to high (80%–90%) (FAO 2018). Mortalities have been reported in juveniles and adults. Early developmental stages of tilapia (fertilized eggs, yolk-sac fish and fry) have also tested positive (Dong et al. 2017a).</p> <p>TiLV has spread rapidly since it was initially reported in Israel in 2009 and is now confirmed in at least 16 countries on three continents (Africa, Asia and South America). Because live tilapia are highly traded internationally, it is probably also present in a many other countries.⁷</p> <ul style="list-style-type: none"> • In Africa: Angola, Egypt, Ghana (in co-infections with ISKNV and <i>Streptococcus agalactiae</i> 1b), Israel and Lake Victoria, which borders Uganda, Kenya and Tanzania (FAO 2018; Reantaso 2020; Lee and Arnaud 2020). • In South America: Colombia, Ecuador and Peru (FAO 2018). It is now known that the cause of tilapia syncytial hepatitis in Ecuador is TiLV. • In Asia: Bangladesh, India, Indonesia Malaysia, the Philippines, Thailand and Taiwan (Baoprasertkul 2020; FAO 2020; Somga 2020). • In North America: detected in juvenile tilapia imported into the US and subsequently eradicated (Hartman 2020). Also reported from Mexico (Debnath et al. 2020). <p>An expert knowledge elicitation risk assessment for TiLV (FAO 2018) estimated that the overall risk to Africa from this pathogen is "high." Nigeria was included on a list of 43 countries potentially at risk to TiLV due to importation of tilapia fry from known infected countries.</p> <p>In February 2018, an outbreak of disease due to TiLV caused high mortality in GIFT housed in an outdoor pond at the WorldFish facility in Penang, Malaysia. Using data collected during this outbreak, Barria et al. (2020) reported significant and high host resistance to TiLV in a Nile tilapia breeding population of GIFT origin. These results highlight the significant potential of harnessing selective breeding to improve host resistance to TiLV in farmed Nile tilapia populations.</p>

Pathogen	Infects Nile tilapia?	Infects Nile tilapia fry?	Causes significant disease?	Present in WorldFish facility?	Present in Nigeria?	Further consideration required?	Comments
Infectious spleen and kidney necrosis virus (ISKNV)	Yes	Yes	Yes	Possible	Possible	Yes	<p>ISKNV, an iridovirus, is the type species of the genus <i>Megalocytivirus</i> and causes disease in a range of freshwater and marine fish.</p> <p>ISKNV is closely related to red sea bream iridovirus (RSIV), and both viruses are notifiable to the OIE (2019a). The OIE (2019a) considers ISKNV distinct from RSIV but as just one of the viruses causing RSIV. In addition to mandarin fish, the OIE (2019b) also lists red drum, flathead mullet and <i>Epinephelus</i> sp. as susceptible to ISKNV.</p> <p>Because of the many similar viruses, the host and geographical range of ISKNV remains unclear, and summaries often list ISKNV as being among the RSIV-like viruses.⁸</p> <p>Although the OIE does not list tilapia as a susceptible species, recent reports from the US and Thailand suggest that it is susceptible and likely to suffer significant mortality (Ramírez-Paredes et al. 2021).</p> <p>ISKNV appears to be an emerging pathogen with the potential to severely impact tilapia culture in Africa and elsewhere. Ramírez-Paredes et al. (2021) have recently reported very high mortality (>50% production) in intensive tilapia cage culture systems across Lake Volta in Ghana. Affected Nile tilapia showed darkening, erratic swimming and abdominal distension with associated ascites. Histopathological observations of tissues taken from moribund fish revealed lesions indicative of viral infection. These included hematopoietic cell nuclear and cytoplasmic pleomorphism with marginalization of chromatin and fine granulation.</p>
Bohle iridovirus (BIV)	Possible	Possible	Yes	No	No	No	<p>BIV is a <i>Ranavirus</i> that was first isolated from ornate burrowing frogs that died at the time of metamorphosis. Although rarely recognized in natural disease, experimental studies indicate that BIV is a potential pathogen of fish, amphibians and reptiles.</p> <p>Areil and Owens (1997) reported periodic mortalities reaching 100% over a period of 60 days in <i>O. mossambicus</i> fry. Moribund fish exhibited rapid corkscrew-like swimming patterns, and the syndrome was successfully transmitted via cannibalism to naïve populations of tilapia fry. BIV-infected tilapia and those succumbing to “spinning tilapia syndrome” shared similarities in histopathological lesions of kidney and muscle. The barramundi <i>Lates calcarifer</i> bioassay indicated that the etiological agent of the epizootic was BIV.</p> <p>The geographic distribution of BIV is restricted to Australia, with reports of infection limited to an Australian aquatic laboratory (Johnston 2008).</p>

Pathogen	Infects Nile tilapia?	Infects Nile tilapia fry?	Causes significant disease?	Present in WorldFish facility?	Present in Nigeria?	Further consideration required?	Comments
Spring viremia of carp virus (SVCV)	Yes [?]	Possible	Yes	Possible	No	Yes	<p>SVCV is a member of the genus <i>Vesiculovirus</i> in the family <i>Rhabdoviridae</i>. It is the cause of spring viremia in carp (SVC), an OIE-listed disease causing an acute hemorrhagic and contagious viremia in several carp species and some other cyprinids and ictalurids.</p> <p>Although SVCV was reported to have been isolated from Nile tilapia from Egypt by Soliman et al. (2008), the OIE (2019b) notes that immuno-histochemistry constituted the sole basis for this identification; electron microscopy purported to show the virus in the nucleus, which is not a feature of SVCV infection. Ibrahim (2020) suggested that Nile tilapia is more resistant to SVCV than the susceptible hosts (Cyprinidae). However, under stressful conditions leading to immunosuppression, it can be infected with the virus and serve as a carrier. There have been no subsequent reports of isolation of SVCV from tilapia or other cichlids. As such, the ability of SVCV to infect Nile tilapia requires confirmation.</p> <p>SVCV has not been reported from Southeast Asia or West Africa. The OIE (2019b) notes that its confirmed distribution includes most European countries, and certain Independent States of the former Soviet Union (Belarus, Georgia, Lithuania, Moldova, Russia and the Ukraine), Brazil, the US, Canada and China.</p> <p>Confirmation of the isolation of SVCV from rainbow trout and Indian carp in Iran, and from Nile tilapia in Egypt awaits more data.</p>
Tilapia larval encephalitis virus (TLEV)	Possible	Possible	Yes	No	No	No	<p>TLEV is a herpes-like virus (family <i>Herpesviridae</i>) causing viral encephalitis of tilapia larvae, characterized by neurological signs and increased mortality of laboratory-reared larvae of blue tilapia (<i>O. aureus</i>). It has only been reported from Israel (Shlapobersky et al. 2010); there have been no reports of similar neurological conditions elsewhere and no identification of this virus outside Israel.</p>
Aquabirnavirus (IPNV-like virus)	Possible	Possible	Yes	Possible	Possible	Yes	<p>Infectious pancreatic necrosis virus (IPNV) is the type species of the genus <i>Aquabirnavirus</i> (family <i>Birnaviridae</i>) and is the cause of infectious pancreatic necrosis, an acute and highly contagious disease of juvenile salmonids.</p> <p>According to Dopazo (2020), the term IPNV is strictly used for those strains affecting salmonids that develop specific clinical signs. Where the virus affects non-salmonids, with different clinical signs, the term "IPNV-like" is applied. In general terms, the name aquabirnaviruses is employed.</p> <p>Aquabirnaviruses are widespread in the aquatic environment and are known to infect cichlids (Johnston 2008). Tilapia have been shown to be experimentally susceptible to birnaviruses, and an <i>Aquabirnavirus</i> related to the Ab serotype of IPNV was isolated from <i>O. mossambicus</i> in Taiwan.</p> <p>Mulei et al. (2018) have recently reported infections of IPNV in cultured rainbow trout and tilapia in Kenya that are identical to European isolates; however, infections were not associated with any clinical signs of disease.</p> <p><i>Aquabirnavirus</i> has been reported from <i>O. mossambicus</i> and <i>Tilapia</i> spp., so it is likely that Nile tilapia is susceptible to infection.</p>

Pathogen	Infects Nile tilapia?	Infects Nile tilapia fry?	Causes significant disease?	Present in WorldFish facility?	Present in Nigeria?	Further consideration required?	Comments
Epizootic hematopoietic necrosis virus (EHNV)	No	No	Yes	No	No	No	<p>Epizootic hematopoietic necrosis is an OIE-listed disease caused by EHNV, a member of the genus <i>Ranavirus</i> in the family <i>Iridoviridae</i>. Since the recognition of this disease due to EHNV in Australia in 1986, similar systemic necrotizing iridovirus syndromes have been reported in farmed fish from several countries in Europe.</p> <p>Tilapia are not included among the species that fulfill the criteria for listing as susceptible to infection with EHNV according to the OIE's Aquatic Animal Health Code (OIE 2019a). Tilapia and other cichlids have not been reported as either susceptible to EHNV or as having evidence of susceptibility.</p> <p>This specific iridovirus has been reported only from Australia.</p>
Carp edema virus (CEV)	No	No	Yes	Possible	Possible	No	<p>CEV, which belongs to the family <i>Poxviridae</i>, causes viral edema of carp, which was first reported in Japan in the 1970s. Clinical signs of viral edema of carp include lethargy, anorexia, skin lesions, enophthalmos, edema and gill necrosis. Mortality of up to 80%–100% may occur within 2–3 weeks of disease (Kim et al. 2020).</p> <p>CEV has been spread globally, due to subclinical infections that are undetectable during disease screening, and has caused outbreaks in numerous European and Asian countries.</p> <p>Infections in Nile tilapia and other cichlid fish have not been reported.</p>
Koi herpesvirus (KHV)	Yes	Possible	Yes	Possible	Possible	Yes	<p>KHV is the cause of koi herpesvirus disease, a serious disease of common and koi carps (<i>Cyprinus carpio</i>), and is listed by the OIE (2019a).</p> <p>A recent study conducted in East Java, Indonesia, by Wahidi et al. (2019) has shown by PCR the presence of KHV in Nile tilapia co-cultured with common carp. Clinical signs observed in tilapia included skin discoloration and white patches on the gills. Genetic variations clearly indicated that the KHV infecting tilapia is an Asian genotype. However, although tilapia was infected with KHV, no specific clinical signs and no mortality was seen in this fish.</p> <p>The OIE (2019b) lists a number of non-cyprinid fish species for which pathogen-specific positive (PCR) results have been reported. However, an infection has not been demonstrated, so the results of the study by Wahidi et al. (2019) could simply represent the detection of viral particles being shed by the co-cultured common carp.</p>
Tilapia parvovirus (TiPV)	Yes	Possible	Yes	No	No	Yes	<p>TiPV was recently described by Liu et al. (2020) in the new genus <i>Chapparvovirus</i>.</p> <p>In 2015, a massive mortality in cage-farmed Nile tilapia occurred in China. This disease appeared to be highly contagious and lethal to all sizes of cage-cultured adult tilapia, and mortality reached 60%–70%. Clinical signs included lethargy, anorexia and a change in swimming behavior that included darting or corkscrew movements. Diseased fish presented hemorrhages on the body surface, lower jaw, anterior abdomen and fin bases, along with exophthalmia and pronounced ocular lesions.</p> <p>Although so far only known from China, TiPV appears to be an emerging viral pathogen with implications for culture of tilapia in China and elsewhere.</p>

Pathogen	Infests Nile tilapia?	Infests Nile tilapia fry?	Causes significant disease?	Present in WorldFish facility?	Present in Nigeria?	Further consideration required?	Comments
Viral nervous necrosis virus (VNNV)	Yes	Yes	Yes	No	No	Yes	<p>Viral encephalopathy and retinopathy (VER), otherwise known as VNN, is an OIE-listed disease. It is a serious disease of several marine fish species and is characterized by significant losses associated with vacuolating lesions of the central nervous system and the retina (OIE 2019b).</p> <p><i>Betanodavirus</i>, the cause of VNN, has been recorded in many cultured marine fish species worldwide and more recently from freshwater species, causing high mortalities, especially in larval and juvenile fish. VNN is reported to infect Nile tilapia, so there is a possibility that tilapia can be carriers or reservoirs of this virus (OIE 2019b).</p> <p>A <i>Betanodavirus</i> has been associated with a mass mortality of larval Nile tilapia maintained in freshwater at 30°C. Although a phylogenetic clustering of this isolate within the red-spotted grouper NNV type was found, the tilapia isolate formed a unique branch distinct from other betanodavirus isolates (Bigarré et al. 2009).</p> <p>More recently, Prihartini et al. (2015) have described a <i>Betanodavirus</i> infection in randomly sampled juvenile tilapia (<i>Oreochromis</i> sp.) from Central Java, Indonesia. The study showed a clear pathogenomic VNN, such as vacuolization and inclusion body in the brain and eyes, similar to the pattern of infection in naturally infected marine fish. By phylogenetic analysis, the isolates from tilapia seed belonged to the red grouper nervous necrosis virus (RGNNV) genotype. Although no clinical signs of disease were observed, this study shows the possibility that tilapia could be a carrier or reservoir of this virus.</p>
Lymphocystis	Possible	Possible	No	No	Yes	No	<p>Lymphocystis is a chronic disease of freshwater and marine fish caused by infection with an iridovirus known as <i>Lymphocystivirus</i> or lymphocystis disease virus (LCDV), which is a member of the family <i>Iridoviridae</i>. Infection causes pebble or wart-like nodules most commonly seen on the fins, skin or gills. Although lymphocystis normally does not cause significant mortalities, it does cause unsightly growths on fish that reduce their marketability. In some cases, severely infected fish may die. Lymphocystis has been reported in over 125 different marine and freshwater fish species from 34 different families, including cichlids (Yanong 2010).</p> <p>In Africa, lymphocystis has only been identified in cichlids, including species of <i>Tilapia</i>, <i>Oreochromis</i> and <i>Haplochromis</i> in East Africa (Paperna 1996). It has also been reported from Nigeria (Okaeme et al. 1988).</p>
BACTERIA							
Globally, there are no bacteria infecting Nile tilapia or other cichlids that are not ubiquitous in freshwater aquatic environments (Dr. Iddiya Karunasagar, personal communication, 2020). Therefore, because all species are opportunistic pathogens that can be expected to be present in the aquatic environments of Nigeria, they will not be considered further in this risk assessment. Below are the main species isolated from Nile tilapia and reported to have caused disease in aquaculture. ⁹							
<i>Actinomyces</i> spp.							This causes rust-yellow skin discoloration in <i>O. niloticus</i> and <i>C. zillii</i> .
<i>Aeromonas</i> spp. <i>A. hydrophila</i> <i>A. caviae</i> <i>A. sobria</i>							<i>Aeromonas hydrophila</i> , <i>A. caviae</i> and <i>A. sobria</i> are the cause of motile aeromonas septicemia. Clinical signs, pathology and associated morbidity and mortality depend on the strain and can range from per-acute with high mortality among young fish to chronic (ulcerative) infections (Ibrahim 2020).
<i>Aeromonas salmonicida</i>							<i>Aeromonas salmonicida</i> subsp. <i>salmonicida</i> is the etiological agent of furunculosis in salmonids and a cause of ulcer disease in cyprinids and marine flatfish. Atypical <i>A. salmonicida</i> was isolated from a single farmed <i>O. niloticus</i> as well as several marine fish species from Oman (Alghabshi et al. 2018). However, laboratory experiments using rainbow trout and Nile tilapia produced no evidence of pathogenicity among the isolates. <i>Aeromonas salmonicida</i> has also been reported from Nile tilapia in Columbia (Grajales-Hahn et al. 2019). Tilapia may thus be considered potential carriers of this bacterium.

Pathogen	Infected Nile tilapia?	Infected Nile tilapia fry?	Causes significant disease?	Present in WorldFish facility?	Present in Nigeria?	Further consideration required?	Comments
<i>Edwardsiella tarda</i>							<p>This species is ubiquitous and widespread globally, although it is considered exotic to some countries.</p> <p><i>Edwardsiella tarda</i> causes edwardsiellosis in freshwater and marine fish species globally and mass mortalities in fish and gastro-intestinal infections in humans. This bacterium has been reported to cause economically significant mortalities in Nile tilapia in different geographical areas (Ibrahim 2020).</p> <p>It has also been reported as an opportunistic pathogen causing septicemia in <i>O. niloticus</i> in Brazil and noted by Johnston (2008) to have serious consequences and zoonotic potential. It has also been reported in <i>O. mossambicus</i>.</p>
<i>Epitheliocystis</i>							<p>Epitheliocystis is a condition affecting the gills and skin of fish and has been reported from more than 90 freshwater and marine species. It is caused by intracellular Gram-negative bacteria, most commonly belonging to the phylum Chlamydiae; however, non-chlamydial bacteria have also been implicated (Seth-Smith et al. 2016).</p> <p>Mortalities have been associated with infections in cultured fish, and it is the cause of gill lesions in <i>O. niloticus</i>.</p>
<i>Flavobacterium columnare</i>							<p>Like other bacteria infecting tilapia, <i>F. columnare</i> is ubiquitous in freshwater environments globally. It is an opportunistic pathogen of fish, causing skin and fin lesions and columnaris disease (cotton-wool disease). As with most fish-infecting bacteria, disease is often the result of environmental and other stress factors, such as high stocking density, high levels of ammonia and organic load (Ibrahim 2020).</p>
<i>Mycobacterium</i> spp.							<p>Mycobacteriosis (fish tuberculosis), a subacute to chronic granulomatous disease, has been reported from Nile tilapia in Kenya and Egypt (Ibrahim 2020).</p> <p>In Mexico, <i>M. fortuitum</i> and <i>M. marinum</i> were associated with massive mortality of Nile tilapia in 2008 (Lara-Flores et al. 2014).</p>
<i>Piscirickettsia</i> -like organisms (PLOs)							<p>These are intercellular bacteria. PLOs have wreaked havoc on tilapia farms in Hawaii and, especially, Taiwan, where farms suffered mortalities of up to 95%.</p> <p>PLOs have been reported in various <i>Oreochromis</i> and <i>Tilapia</i> spp., including <i>O. niloticus</i>. The pathogen appears to be widely distributed in tilapia culture, having been reported in Taiwan, Japan, Jamaica, Indonesia, Central America and the US (Johnston 2008). Most reports of PLOs appear to involve intracellular bacteria belonging to the genus <i>Francisella</i>.</p>
<i>Pasteurella multocida</i>							<p>This bacterium is a cause of septicemia. Infections in fish are extremely rare, with only one reported case in hybrid tilapia (<i>O. aureus</i> x <i>O. niloticus</i>) in Israel.</p>
<i>Plesiomonas shigelloides</i>							<p>This species has reportedly caused mortality in fry of <i>Oreochromis</i> spp. in Taiwan.</p>
<i>Providencia rettgeri</i>							<p>This bacterium was isolated from the kidney of <i>O. niloticus</i> in Egypt.</p>
<i>Pseudomonas</i> spp. <i>P. fluorescens</i> <i>P. putida</i>							<p>These species can cause chronic infection and acute septicemia.</p> <p>In Egypt, <i>Pseudomonas</i> spp. were isolated from 30.8% of lake-cultured <i>O. niloticus</i> examined in association with a disease outbreak; during the episode of mass mortality, some fish showed signs typical of <i>Pseudomonas</i> septicemia (Ibrahim 2020).</p>
<i>Staphylococcus epidermidis</i> ; <i>Staphylococcus</i> sp.							<p>These bacteria have caused septicemia and mass mortalities of <i>Oreochromis</i> spp. in Taiwan and Egypt.</p>

Pathogen	Infected Nile tilapia?	Infected Nile tilapia fry?	Causes significant disease?	Present in WorldFish facility?	Present in Nigeria?	Further consideration required?	Comments
<i>Streptococcus</i> spp. <i>S. agalactiae</i> 1b <i>S. iniae</i>							<p><i>Streptococcus</i> is considered the most economically important disease of Nile tilapia. It has led to private-sector efforts to develop <i>Streptococcus</i>-resistant strains of <i>O. niloticus</i>.¹⁰</p> <p>Verner-Jeffreys et al. (2018), who conducted a comprehensive disease investigation in tilapia farmed in Lake Volta, Ghana, found that <i>S. agalactiae</i> multilocus sequence type 261 was a major cause of mortality.</p> <p><i>Streptococcus iniae</i> has been reported from at least 27 species of freshwater and marine fish and is a major cause of losses in aquaculture, including tilapia culture. Diseased fish display lethargy and dark coloration, with variable degrees of other signs of septicemia (ascites, eye hemorrhage, skin hemorrhage, behavioral changes etc.). This species is widely distributed geographically, but apparently exotic to some countries (e.g. New Zealand).</p>
<i>Vibrio</i> spp. <i>V. anguillarum</i> <i>V. ordalii</i> <i>V. damsela</i> <i>V. vulnificus</i>							<p><i>Vibrio</i> spp. are ubiquitous globally in both freshwater and marine environments. In Egypt, <i>V. vulnificus</i> was noted to cause septicemia and ulceration in <i>Oreochromis</i> spp., while <i>V. anguillarum</i>, as the cause of economically damaging infectious disease, was isolated from 62% of clinically affected Nile tilapia. The latter was also associated with mass mortalities (nearly 98%) of Nile tilapia compromised by exposure to extreme cold water (5.2°C) at a farm in the north of the country (Ibrahim 2020).</p>
<i>Yersinia ruckeri</i>							<p><i>Yersinia ruckeri</i> is ubiquitous globally in freshwater environments and a cause of bacterial septicemia in <i>O. niloticus</i> and other fish species.</p>
PROTISTA							
Flagellata							
<i>Cryptobia branchialis</i>, <i>Cryptobia</i> spp.	Yes	Yes	Possible	Possible	Yes	Yes	<p>Most species are ubiquitous and non-pathogenic commensals on the gills of freshwater and marine fish. However, the freshwater species <i>C. branchialis</i>, reported from Nile tilapia in the Philippines (Arthur and Lumanlan-Mayo 1997) is associated with the disease "gill cryptobiosis." <i>Cryptobia iubilans</i>, which infects the intestine of cichlid fish (Lom and Dyková 1992) is also reported to be pathogenic. The latter species has been reported from Nigeria in catfish (see Omeji et al. 2011; Iyabo et al. 2015)</p> <p>This genus is listed as infecting <i>O. niloticus</i> in Africa (Scholz et al. 2018), and an unidentified <i>Cryptobia</i> has been reported from this host in Nigeria (Table 5).</p>
<i>Hexamita</i> spp.	Yes	Yes	Possible	Possible	Yes	Yes	<p>In Nigeria, <i>Hexamita</i> sp. has been reported from <i>S. melanotheron</i> (Table 5), while <i>H. intestinalis</i> has been reported from the catfish <i>Chrysichthys nigrodigitatus</i> by Iyabo et al. (2015).</p> <p>The genus is listed as infecting <i>O. niloticus</i> in Africa (Scholz et al. 2018).</p>
<i>Ichthyobodo necatrix</i>	Yes	Yes	Possible	Yes	Yes	Yes	<p>These are ubiquitous ectoparasites that infect the skin and gills of many freshwater fish species.</p> <p>In Nigeria, <i>I. necatrix</i> has been reported from Nile tilapia and other cichlids by Omoniye and Ojelade (2017), and from the catfish <i>Chrysichthys nigrodigitatus</i> by Iyabo et al. (2015).</p> <p><i>Ichthyobodo necatrix</i> causes ichthyobodosis, a disease affecting mainly young fish that is treatable in enclosed aquaculture facilities (Lom and Dyková 1992).</p>

Pathogen	Infects Nile tilapia?	Infects Nile tilapia fry?	Causes significant disease?	Present in WorldFish facility?	Present in Nigeria?	Further consideration required?	Comments
<i>Piscinoodinium pillulare</i>	Yes	Yes	Yes	Possible	Yes	Yes	<p>This is a ubiquitous ectoparasite affecting the skin and gills of many freshwater fish species, including <i>O. niloticus</i>, <i>C. rendalli</i> and <i>O. mossambicus</i>.</p> <p>In Nigeria, an unidentified <i>Piscinoodinium</i> has been reported from <i>O. niloticus</i> (Table 5), and <i>P. pillulare</i> has been reported from the catfish <i>Chrysichthys nigrodigitatus</i> (Iyabo et al. (2015).</p> <p><i>Piscinoodinium</i> is the cause of piscinoodinosis, a dangerous but treatable disease in aquaria and tropical fish culture (Lom and Dyková 1992).</p>
Ameobida							
<i>Hartmanella vermiformis</i>	Yes	Possible	No	Possible	Possible	No	<i>Hartmanella vermiformis</i> is a common, free-living amoeba that is widespread in nature. It has been isolated from soil, freshwater, air and a variety of engineered water systems (Lom and Dyková 1992). It was also isolated from the kidney tissue of farmed Nile tilapia in the former Czechoslovakia (Dyková et al. 1997).
<i>Mayorella</i> -like & <i>Platyamoeba</i> -like organisms	Yes	Possible	No	Possible	Possible	No	These are free-living amoebae that were reported to infect the liver of farmed Nile tilapia in the former Czechoslovakia (Dyková et al. 1997).
<i>Rosculus ithacus</i>	Yes	Possible	No	Possible	Possible	No	This species is a free-living amoeba that is an occasional opportunistic parasite of snakes and fish. It was isolated from the kidney of farmed Nile tilapia in the former Czechoslovakia (Dyková et al. 1997).
Ciliatea							
<i>Apiosoma minutum</i>	Yes	Yes	No	Possible	Possible	No	<p>This is a ubiquitous commensal found on the skin and gills of freshwater fish.¹¹ Species of this ciliate genus can be expected to be encountered in Nigeria.</p> <p><i>Apiosoma minutum</i> has been reported from Nile tilapia in Vietnam (Arthur and Te 2006).</p>
<i>Cryptocaryon irritans</i>	Yes	Yes	Yes	No	Possible	No	<i>Cryptocaryon</i> is a ubiquitous ciliate parasitic on the gills and skin of many marine fish species. It is the cause of cryptocaryonosis (marine white spot disease), a treatable disease in marine aquaria. It does not occur in freshwater systems.
<i>Chilodonella</i> spp. <i>C. hexasticha</i> <i>C. piscicola</i>	Yes	Yes	Yes	Possible	Yes	Yes	<p><i>Chilodonella</i> spp. infect the skin and gills, and are ubiquitous on many freshwater fish species.</p> <p>This genus is common in hatcheries and other production facilities and may occasionally cause disease in stressed fish, but is treatable.</p> <p><i>Chilodonella hexasticha</i> is listed as occurring on <i>O. niloticus</i> in Africa by Scholz et al. (2018), while in Nigeria, <i>C. uncinata</i> has been reported from <i>Chrysichthys nigrodigitatus</i> (Iyabo et al. 2015) and an unidentified <i>Chilodonella</i> has been reported from <i>O. niloticus</i> (Table 5).</p>
<i>Epistylis</i> spp.	Yes	Yes	No	Possible	Yes	No	<i>Epistylis</i> is a ubiquitous commensal on the skin and gills of freshwater fish. ¹² It has been listed from <i>O. niloticus</i> in Africa (Scholz et al. 2018) and reported from the same host in Nigeria (Table 5).
<i>Ichthyophthirius multifiliis</i>	Yes	Yes	Yes	Possible	Yes	Yes	<p>This pathogenic ciliate infects the skin and gills and is a ubiquitous pathogen of freshwater fish. It has been listed from <i>O. niloticus</i> in Africa by Scholz et al. (2018) and is widely reported from tilapia species in Nigeria.</p> <p>It is a serious pathogen in hatcheries and aquaria, causing "whitespot disease," and is difficult to eradicate from closed systems.</p>

Pathogen	Infects Nile tilapia?	Infects Nile tilapia fry?	Causes significant disease?	Present in WorldFish facility?	Present in Nigeria?	Further consideration required?	Comments
Paratrichodina spp. <i>P. africana</i> <i>P. incissa</i>	Yes	Yes	No	Possible	Possible	No	<i>Paratrichodina africana</i> is listed as infecting <i>O. niloticus</i> in Africa (Scholz et al. 2018), while <i>P. incissa</i> occurs on the same host in Vietnam (Arthur and Te 2006). This genus does not appear to have been associated with disease in tilapia culture.
Tetrahymena spp.	Yes	Yes	Possible	Possible	Yes	Yes	<i>Tetrahymena</i> spp. are saprozoic and occasional pathogens of freshwater fish. <i>Tetrahymena corlissi</i> causes "tet disease" in freshwater fish held in aquaria (Lom and Dyková 1992). An unidentified species of <i>Tetrahymena</i> has been reported from Nile tilapia in Nigeria (Table 5).
Trichodinella spp. <i>T. epizootica</i>	Possible	Possible	No	Possible	Possible	No	This genus has been recorded from the gills of <i>O. mossambicus</i> . <i>Trichodinella epizootica</i> is ubiquitous and shows little host specificity. It is common in hatcheries and other production facilities and may occasionally proliferate massively, causing disease in stressed fish (Lom and Dyková 1992), but it is treatable.
Trichodina spp. <i>T. acuta</i> <i>T. centrostrigata</i> <i>T. cichlidarum</i> <i>T. compacta</i> <i>T. fultoni</i> <i>T. heterodentata</i> <i>T. magna</i> <i>T. migala</i> <i>T. mutabilis</i> <i>T. nigra</i> <i>T. oreochromisi</i> <i>T. orientalis</i> <i>T. pediculus</i> <i>T. rectuncinata</i> <i>T. reticulata</i> <i>T. siluri</i> <i>T. tilapiae</i> <i>T. truttae</i> <i>T. velasquezae</i>	Yes	Yes	Yes	Possible	Yes	Yes	<i>Trichodina</i> is a ubiquitous genus containing more than 100 described species that infect the skin and/or gills and occasionally the urinary bladder of freshwater and marine fish. Certain species many have limited geographical distributions and/or show high host specificity. Globally, at least 16 species are reported from Nile tilapia, with <i>T. acuta</i> and <i>T. heterodentata</i> being reported from <i>O. niloticus</i> in Nigeria (Table 5). Many other species are likely to infect Nigerian freshwater fish. <i>Trichodina</i> spp. are common in hatcheries and other production facilities and occasionally cause trichodinosis in stressed fish, which is a treatable disease. Pathogenicity may vary between parasite and host species.
Tripartiella spp. <i>T. bulbosa</i> <i>T. cichlidarum</i> <i>T. clavodonta</i> <i>T. leptospina</i> <i>T. nana</i> <i>T. obtusa</i> <i>T. orthodens</i> <i>T. spatula</i> <i>T. tilapiae</i>	Yes	Yes	No	Possible	Possible	No	<i>Tripartiella</i> spp. Are ubiquitous and commensal. The genus is common on the gills of freshwater fish, with at least nine species occurring on Nile tilapia.
Trichophrya spp.	Yes	Yes	No	Possible	Possible	No	This ubiquitous ciliate is ectocommensal on the gills of freshwater fish (Lom and Dyková 1992).
Coccidia							
Goussia cichlidarum	Yes	Possible	No	Possible	Possible	No	<i>Goussia cichlidarum</i> is common in the epithelial lining of the swim bladder of tilapia in Israel and Uganda (Lom and Dyková 1992). It has been listed from <i>O. niloticus</i> in Africa (Scholz et al. 2018).

Pathogen	Infects Nile tilapia?	Infects Nile tilapia fry?	Causes significant disease?	Present in WorldFish facility?	Present in Nigeria?	Further consideration required?	Comments
FUNGI							
<i>Aphanomyces invadans</i>	Yes	Possible	Yes	Possible	Possible	Yes	<i>Aphanomyces invadans</i> is the cause of EUS, a serious transboundary disease affecting many species of freshwater fish with near global distribution that is listed by the OIE. Tilapia species are generally considered resistant but are more accurately characterized as having low susceptibility (Johnston 2008) and thus are potential carriers.
<i>Branchiomyces demigrans</i>	Yes	Yes	Yes	No	Possible	No	This non-septate filamentous fungus is an opportunistic pathogen of freshwater fish and has been reported to cause gill rot and mortalities of cultured Nile tilapia in Egypt (Khalil et al. 2015).
<i>Fusarium oxysporum</i>	Possible	Yes	No	No	Possible	No	<i>Fusarium oxysporum</i> has a global distribution and occurs chiefly as a soil saprophyte. An opportunistic pathogen of marine fish causing subcutaneous lesions, <i>F. oxysporum</i> in co-infection with <i>Aeromonas hydrophila</i> has been associated with mortalities in Nile tilapia fry held in freshwater culture in Spain (Cutuli et al. 2015).
<i>Ichthyophonus hoferi</i>	Yes	Possible	Yes	No	Possible	No	Ichthyophonosis, caused by the parasitic fungus <i>I. hoferi</i> is an important disease of marine and anadromous fish. It has a broad host range and global distribution (El-Ghany and Alla 2008; Kocan 2018). It is listed by Johnston (2008) as reported from <i>O. niloticus</i> . Infections range from subclinical, with no gross or microscopic signs of disease, to clinical disease that can present as external ulcers, extensive tissue and organ damage and heavy mortality (Kocan 2018).
<i>Loma camerouensis</i>	Yes	Possible	Possible	No	No	No	The genus <i>Loma</i> belongs to the Microsporida, which were formerly considered protozoans but are now recognized as fungi. <i>Loma camerouensis</i> was reported from xenomas in the gut of cultured <i>O. niloticus</i> in Cameroon by Fomena et al. (1992).
<i>Rhizomucor</i> spp.	Possible	Possible	No	No	Possible	No	<i>Rhizomucor</i> is a genus of saprophytic fungus that is ubiquitous in the environment but is considered a rare opportunistic pathogen of freshwater fish. It is listed by Johnston (2008) from the skin of <i>O. niloticus</i> x <i>O. aureus</i> x <i>O. mossambicus</i> from the US.
<i>Saprolegnia</i> spp.	Yes	Yes	No	No	Yes	No	<i>Saprolegnia</i> spp. are ubiquitous and opportunistic pathogens of freshwater fish. In Nigeria, several species of this genus have been associated with infections in freshwater fish species (Ogbonna and Alabi 1991). Johnston (2008) listed <i>Saprolegnia</i> sp. from the skin of <i>O. niloticus</i> and <i>O. aureus</i> from Egypt.
MONOGENEA							
<i>Cichlidogyrus</i> spp. <i>C. aegypticus</i> <i>C. arthracanthus</i> <i>C. cirratus</i> <i>C. doussoui</i> <i>C. halli</i> <i>C. haplochromi</i> <i>C. levequei</i> <i>C. mbirizei</i> <i>C. nematocirrus</i> <i>C. rognoni</i> <i>C. sclerosus</i> <i>C. thurstonae</i> <i>C. tiberianus</i> <i>C. tilapiae</i> <i>C. tubicirrus magnus</i>	Yes	Yes	No	Possible	Possible	No	This is a large genus containing some 125 described species, almost all of which are only known from cichlid fish (le Roux and Avenant-Oldewage 2010; Gereates et al. 2020). The species listed herein are only those that have been reported from Nile tilapia. Many of these species are undoubtedly present in Nigeria. <i>Cichlidogyrus</i> spp. are gill parasites with a direct life cycle. They have often been translocated to new geographic areas along with the movement of infected tilapia for aquaculture development. Members of this genus do not appear to have been reported to cause problems in aquaculture, although their potential pathogenicity and the possibility of host switching from introduced to native species has been mentioned (Gereates et al. 2020). As with other monogeneans, infections in enclosed aquaculture facilities should be treatable.

Pathogen	Infects Nile tilapia?	Infects Nile tilapia fry?	Causes significant disease?	Present in WorldFish facility?	Present in Nigeria?	Further consideration required?	Comments
Dactylogyrus spp. <i>D. extensus</i>	Yes	Possible	Yes	Possible	Yes	Yes	<p>The genus <i>Dactylogyrus</i> has occasionally been reported to infect Nile tilapia. However, species identifications have not been made, and most or all of these records may involve the misidentification of <i>Cichlidogyrus</i> spp.</p> <p><i>Dactylogyrus extensus</i> has been reported from Nile tilapia in Nigeria (Table 5) and there are also several reports of unidentified <i>Dactylogyrus</i> spp. from tilapia species.</p>
Enterogyrus spp. <i>E. cichlidarum</i> <i>E. coronatus</i> <i>E. hemi-haplochromii</i> <i>E. malmbergi</i> <i>E. niloticus</i>	Yes	Possible	No	Possible	Possible	No	<p><i>Enterogyrus</i> spp. are parasites in the stomach of cichlid fish, with 12 species currently described within this genus (Madanire-Moyo and Avenant-Oldewage 2014; Luus-Powell et al. 2020).</p> <p>They are host specific to cichlids and have often been translocated to new geographic areas along with the movement of infected tilapia for aquaculture development.</p> <p><i>Enterogyrus</i> spp. infecting Nile tilapia do not appear to cause problems in aquaculture. Madanire-Moyo and Avenant-Oldewage (2015) found that histopathological changes caused by the <i>E. coronatus</i> in the stomach of wild <i>Pseudocrenilabrus philander</i> were mild and restricted to the vicinity of haptor attachment.</p> <p>It is possible that veterinary drugs such as ivermectin that are used to treat internal parasites may be effective against these monogeneans, but this has not yet been investigated.</p>
Gyrodactylus spp. <i>G. cichlidarum</i> (syn.: <i>G. niloticus</i>) <i>G. elegans</i> <i>G. ergensi</i> <i>G. hildae</i> <i>G. malalai</i> <i>G. nyanzae</i> <i>G. occupatus</i> <i>G. parisellei</i> <i>G. shariffi</i> <i>G. sprostonae</i> <i>G. yacatli</i>	Yes	Yes	Yes	Possible	Possible	Yes	<p>The genus <i>Gyrodactylus</i> comprises some 409 potentially valid species (Harris et al. 2004). Many species are host specific. <i>Gyrodactylus</i> spp. are viviparous.</p> <p><i>Gyrodactylus</i> spp. are frequent parasites of the skin and gills of freshwater fish, with members of this genus often causing problems in fish cultured in closed systems.</p> <p><i>Gyrodactylus</i> spp. have often been translocated to new geographic areas along with the movement of infected fish for aquaculture development. García-Vásquez et al. (2011) noted that <i>Gyrodactylus</i>-associated mortality of juvenile, pond-reared <i>O. niloticus</i> has been reported across several continents and stressed that extreme caution should be exercised in the translocation of commercial tilapiine species into areas where cichlids are already resident.</p> <p>Scholz et al. (2018) listed seven species occurring on Nile tilapia in Africa.</p> <p>As with other monogeneans infecting the gills or skin of freshwater fish cultured in closed systems, infections are treatable.</p>
Macrogyrodactylus spp.	Yes[?]	?	No	No	Yes	No	<p><i>Macrogyrodactylus</i> spp. are enzootic to Africa and have been described mainly from the gills and fins of <i>Clarias</i> and <i>Polyopterus</i> (Prikrylová and Gelnar 2008). The report of an unidentified species from Nile tilapia in Nigeria by Ashade et al. (2010) requires confirmation, as it appears to be the only finding of this genus on a cichlid fish.</p>
Scutogyrus spp. <i>S. longicornis</i> (syn.: <i>C. longicornis</i> , <i>C. longicornis longicornis</i>) <i>S. minus</i>	Yes	Possible	No	Possible	Possible	No	<p>This genus is closely related to <i>Cichlidogyrus</i> and shares many of its characteristics. Two species have been reported from <i>O. niloticus</i> in Africa (Scholz et al. 2018).</p>

Pathogen	Infects Nile tilapia?	Infects Nile tilapia fry?	Causes significant disease?	Present in WorldFish facility?	Present in Nigeria?	Further consideration required?	Comments
CRUSTACEA							
Brachiura							
<i>Argulus</i> spp. <i>A. africanus</i> <i>A. japonicus</i> <i>A. rhipidiophorus</i>	Yes	Yes	Yes	No	Yes	No	Scholz et al. (2018) list two species of <i>Argulus</i> as infecting Nile tilapia in Africa, while <i>A. africanus</i> has been reported from this host in Nigeria (Table 5). <i>Argulus</i> spp. are macroscopic parasites on the skin of freshwater fish and are easily seen by hatchery workers.
<i>Dolops ranarum</i>	Yes	Possible	Possible	No	Possible	No	This brachiuran was listed from <i>O. niloticus</i> in Africa by Scholz et al. (2018). Closely related to <i>Argulus</i> , these macroscopic parasites are easily seen in hatchery situations.
Copepoda							
<i>Caligus epidemicus</i>	Yes	Possible	Yes	No	No	No	This is a marine and brackish-water parasite of Nile tilapia raised in brackish water in the Philippines (Arthur and Lumanlan-Mayo 1997). They are macroscopic parasites that are easily seen by aquaculturists.
<i>Ergasilus latus</i>	Yes	Possible	Possible	Possible	Yes	No	<i>Ergasilus</i> spp. are parasitic copepods that infect the gills of freshwater fish. Heavy infections can cause gill pathology and ergasilosis. <i>Ergasilus latus</i> is primarily a parasite of cichlid fish and is known only from Africa. It has been reported from <i>O. niloticus</i> and other tilapia in Nigeria (Table 5).
<i>Lamproglena monodi</i>	Yes	Possible	No	No	Yes	No	<i>Lamproglena monodi</i> is a copepod parasitic on the gills of cichlid fish. Originally described from cichlids from the Democratic Republic of Congo, this species has also been reported in Egypt, Zimbabwe, Namibia and Uganda, and recorded from Nile tilapia in Nigeria (Table 5). It was recently introduced to Brazil, where it was recorded from Nile tilapia and other species (Kozłowski de Azevedo et al. 2012; Hassan et al. 2013). Parasitic on the skin and gills, it is a macroscopic parasite that is easily seen in closed aquaculture systems. It reportedly does not cause serious gill pathology or mortalities in cichlids (Hassan et al. 2013).
<i>Lernaea</i> spp. <i>L. barnimiana</i> <i>L. cyprinacea</i> <i>L. hardingi</i> <i>L. tilapiae</i>	Yes	Yes	Yes	No	Yes	Yes	Anchor worms (<i>Lernaea</i> spp.) are common parasites of freshwater fish. <i>Lernaea cyprinacea</i> has been reported from Nile tilapia in Nigeria (Table 5). Although highly pathogenic, especially to young fish, these macroscopic parasites are easily seen in hatchery situations, where they may cause lernaeciasis and mortalities. Some species may show host specificity.
<i>Lernaeocera branchialis</i>	Yes	Possible	Yes	No	Yes	No	This pathogen is a marine species of parasitic copepod that has been reported from Nile tilapia in Nigeria (Table 5).
<i>Opistholernaea laterobranchialis</i>	Yes	Possible	Yes	No	Possible	Yes	<i>Opistholernaea laterobranchialis</i> is listed as a parasite of <i>O. niloticus</i> in Africa (Scholz et al. 2018). These are macroscopic parasites that are easily seen in hatchery situations. Infections in mouthbreeding cichlids are reported to impair breeding (Paperna 1996).

Pathogen	Infects Nile tilapia?	Infects Nile tilapia fry?	Causes significant disease?	Present in WorldFish facility?	Present in Nigeria?	Further consideration required?	Comments
Isopoda							
<i>Alitropis typicus</i> , <i>Nerocila orbigny</i>	Yes	Yes	Yes	Possible	No	No	These are large parasites found in the buccal cavity of tilapia and other freshwater fish that are easily detected by aquaculturists. <i>Alitropis typicus</i> is reported to have caused mortalities of 40%–80% in Nile tilapia cultured in net cages in the Philippines, with only 5% of juveniles in Taal Lake surviving infection (Arthur and Lumanlan-Mayo 1997). <i>Nerocila orbigny</i> has been reported from tilapia in Egypt (Ibrahim 2020).
HIRUDINEA							
<i>Piscicola geometra</i>	Yes	Yes	Yes	No	Yes	No	This leech is a widespread European parasite of freshwater fish. It has been reported from Nile tilapia in a rainforest pond in southeastern Nigeria by Opara (2002). The macroscopic size of this leech and the fact that it must leave its fish host to lay cocoons on substrate would make it easily detected in closed aquaculture facilities.
MOLLUSCA							
<i>Cristaria plicata</i>	Yes	Yes	Yes	No	Possible	No	Glochidia of <i>C. plicata</i> have been reported as temporary parasites on the gills of Nile tilapia in the Philippines (Arthur and Lumanlan-Mayo 1997). Adults are free-living freshwater clams.

Table 7. Possible pathogens of Nile tilapia having direct life cycles and their screening against the criteria listed in Section 8.1.

Viruses

Viruses are the most serious threats to tilapia culture. The following seven are given further consideration due to their pathogenicity and the potential harm their introduction might cause to Nigerian aquaculture and wild fish populations:

OIE-listed viruses

- Koi herpesvirus (KHV)
- Viral nervous necrosis virus (VNNV)
- Spring viremia of carp virus (SVCV)

Other viruses

- Tilapia lake virus (TiLV)
- Infectious spleen and kidney necrosis virus (ISKNV)
- Tilapia parvovirus (TiPV)
- *Aquabirnavirus* (IPNV-like virus)

Bacteria

Globally, there are no bacteria infecting Nile tilapia that are not ubiquitous in freshwater aquatic environments. Species causing disease in Nile

tilapia culture, such as *Aeromonas hydrophila*, *Streptococcus agalactiae* and *S. iniae*, are globally distributed. As previously noted, all bacteria considered in this risk analysis have either reportedly been found or are expected to be present in Nigeria; thus, bacterial pathogens are not considered further in this risk assessment.

Fungi

Most of the fungi causing disease in tilapia culture are opportunistic species present in freshwater environments and have broad geographic distributions. One serious fungal disease requires further consideration:

- *Aphanomyces invadans* (epizootic ulcerative syndrome, EUS)

Protista

Eight protozoan taxa that have direct life cycles and are pathogenic to Nile tilapia and other freshwater fish require further consideration:

- *Cryptobia* spp.
- *Hexamita* spp.
- *Ichthyobodo necatrix*

- *Piscinoodinium pillulare*
- *Chilodonella* spp.
- *Ichthyophthirius multifiliis*
- *Tetrahymena* spp.
- *Trichodina* spp.

Monogenea

Two monogenean taxa require further consideration:

- *Dactylogyrus* spp.
- *Gyrodactylus* spp.

Crustacea

Two parasitic copepods require further assessment:

- *Lernaea* spp.
- *Opistholernaea laterobranchialis*

8.4. Calculation of likelihood of pathogen entry and exposure¹³

The following eight-step pathway required for a potential pathogen to enter Nigeria, escape from quarantine and become established in cultured or wild fish populations has been used to estimate likelihood of entry and exposure (Table 8):

Entry

1. Pathogen is present in G17 broodstock.
2. Pathogen escapes detection in G17 broodstock.
3. Pathogen is transferred to G17–F1 fry and establishes infection.
4. Pathogen is present in the subpopulation of G17–F1 fry selected for transfer.
5. Pathogen survives transfer to Nigeria.

Exposure

6. Pathogen is not detected by diagnostic testing of G17–F1 or subsequent generations.
7. Pathogen escapes the Nigerian biosecure quarantine facility due to a lapse in biosecurity or remains undetected and is released from the facility via fry transferred to the NBCs.
8. Pathogen contacts and infects cultured or wild fish populations and becomes established in Nigeria.

Table 8 presents the results of estimation of likelihood of entry (L_{Ent}), likelihood of exposure (L_{Exp}) and likelihood of both entry and exposure ($L_{Ent} \times L_{Exp}$) occurring for the 20 hazards.

The results of this phase of the risk assessment revealed negligible likelihood of entry (L_{Ent}) for 3 hazards (1 virus and 2 crustaceans), very low L_{Ent} for 7 hazards (6 viruses and 1 fungus), and moderate L_{Ent} for 10 hazards (all 8 protozoans and both monogeneans).

The risk assessment then estimated the likelihood of exposure (L_{Exp}) for those 17 hazards having non-negligible L_{Ent} (Table 8). The results showed a very low L_{Exp} for 7 hazards (6 viruses and 1 fungus), and a high L_{Exp} for 10 hazards (all 8 protozoans and both monogeneans).

The risk assessment then proceeded to calculate the likelihood of both entry and exposure ($L_{Ent} \times L_{Exp}$) for the 17 hazards. Seven hazards (all 6 viruses and the fungus) were estimated to have negligible $L_{Ent} \times L_{Exp}$, while the remaining 10 hazards (8 protists and 2 monogeneans) were estimated to have non-negligible $L_{Ent} \times L_{Exp}$.

The next step in the risk assessment process is the estimation of magnitude of the consequence of entry and exposure for the 10 hazards having non-negligible likelihood of entry and exposure ($L_{Ent} \times L_{Exp}$) (Table 9).

The estimated consequence of $L_{Ent} \times L_{Exp}$ was low for all 8 protozoans, low to moderate for 1 monogenean (*Dactylogyrus* spp.), and low to high for the other monogenean (*Gyrodactylus* spp.)

As all 10 hazards were estimated to have non-negligible consequence, they were all taken through the final step in the risk assessment, which is the calculation of their total risk (Table 9). Total risk was estimated as low for all 8 protozoans, low to moderate for *Dactylogyrus* spp., and low to high for *Gyrodactylus* spp.

Pathway step: Hazard (Pathogen)	1	2	3	4	5	L _{Ent}	6	7	8	L _{Exp}	L _{Ent} X L _{Exp}
VIRUSES											
Koi herpesvirus (KHV)	L	VL	H	H	H	VL	VL	L	H	VL	N
Viral nervous necrosis virus (VNNV)	VL	L	H	H	H	VL	VL	L	H	VL	N
Spring viremia of carp virus (SVCV)	VL	H	H	H	H	VL	VL	L	H	VL	N
Tilapia lake virus (TiLV)	VL	VL				N					
Infectious spleen and kidney necrosis virus (ISKNV)	L	VL	H	H	H	VL	VL	L	H	VL	N
Tilapia parvovirus (TiPV)	VL	L	H	H	H	VL	VL	L	H	VL	N
<i>Aquabirnavirus</i> (IPNV-like virus)	VL	L	H	H	H	VL	VL	L	H	VL	N
FUNGI											
<i>Aphanomyces invadans</i> (EUS)	VL	L	H	H	H	VL	VL	L	H	VL	N
PROTISTA											
<i>Cryptobia</i> spp.	M	H	H	H	H	M	H	H	H	H	M
<i>Hexamita</i> sp.	M	H	H	H	H	M	H	H	H	H	M
<i>Ichthyobodo necatrix</i>	M	H	H	H	H	M	H	H	H	H	M
<i>Piscinoodinium pillulare</i>	M	H	H	H	H	M	H	H	H	H	M
<i>Chilodonella</i> spp.	M	H	H	H	H	M	H	H	H	H	M
<i>Ichthyophthirius multifiliis</i>	M	H	H	H	H	M	H	H	H	H	M
<i>Tetrahymena</i> spp.	M	H	H	H	H	M	H	H	H	H	M
<i>Trichodina</i> spp.	M	H	H	H	H	M	H	H	H	H	M
MONOGENEA											
<i>Dactylogyrus</i> spp.	M	H	H	H	H	M	H	H	H	H	M
<i>Gyrodactylus</i> spp.	M	H	H	H	H	M	H	H	H	H	M
CRUSTACEA											
<i>Lernaea</i> spp.	VL	VL				N					
<i>Opistholernaea laterobranchialis</i>	VL	VL				N					

Note: likelihood estimates are combined using Table 3 (N = Negligible, VL = Very Low, L = Low, M = Moderate, H = High); $L_{Ent} = L1 \times L2 \times L3 \times L4 \times L5$, $L_{Exp} = L6 \times L7 \times L8$; Likelihood of Entry and Exposure = $L_{Ent} \times L_{Exp}$.

Table 8. Calculation of estimates of likelihood of entry (L_{Ent}) and exposure (L_{Exp}).

Potential hazard	Estimated likelihood of entry and exposure	Estimated consequence of entry and exposure	Estimated total risk	Remarks
PROTISTA				
<i>Cryptobia</i> spp.	M	L	L	Genus is known to be present in Nigeria (Table 5). Introduction would have only localized effects on receiving aquaculture facilities where infections could be controlled.
<i>Hexamita</i> spp.	M	L	L	Genus is known to be present in Nigeria (Table 5). Introduction would have only localized effects on receiving aquaculture facilities where infections could be controlled.
<i>Ichthyobodo necatrix</i>	M	L	L	Species is known to be present in Nigeria (Table 5). Introduction would have only localized effects on receiving aquaculture facilities where infections could be controlled.
<i>Piscinoodinium pillulare</i>	M	L	L	Genus is known to be present in Nigeria (Table 5). Introduction would have only localized effects on receiving aquaculture facilities where infections could be controlled.
<i>Chilodonella</i> spp.	M	L	L	Genus has been reported from Nigeria (Table 5). Introduction would have only localized effects on receiving aquaculture facilities where infections could be controlled. A recent study by Bu et al. (2021) suggests that <i>C. uncinata</i> is a facultative parasite.
<i>Ichthyophthirius multifiliis</i>	M	L	L	Species has been reported from Nigeria (Table 5). Introduction would have only localized effects on receiving aquaculture facilities where infections could be controlled.
<i>Tetrahymena</i> spp.	M	L	L	Genus is ubiquitous, so it is expected to be present in Nigeria. Introduction would have only localized effects on receiving aquaculture facilities where infections could be controlled.
<i>Trichodina</i> spp.	M	L	L	Genus is ubiquitous and present in Nigeria (Table 5). Introduction would have localized effects on receiving aquaculture facilities where infections could be controlled. However, with the movement of GIFT into Nigeria, the possibility of introducing new species, some of which could be pathogenic to native fish species, should be avoided.
MONOGENEA				
<i>Dactylogyrus</i> spp.	M	L to M	L to M	Although the genus has been reported from Nigeria (Table 5), the potential exists to introduce an exotic species of high pathogenicity, so additional risk management measures are recommended.
<i>Gyrodactylus</i> spp.	M	L to H	L to H	Although the genus has been reported from Nigeria (Table 5), the potential exists to introduce an exotic species of high pathogenicity, so additional risk management measures are recommended.

Table 9. Estimate of total risk for those potential hazards having non-negligible likelihood of entry and exposure ($L_{Ent} \times L_{Exp}$) (calculated using Table 4).

9. Assessment of risk to Nigeria (risk management: risk evaluation)

This risk analysis takes a highly precautionary approach by assuming that the ALOP for Nigeria should be “high,” giving an ALOR that is “low.”

Comparison of estimates of total risk with the suggested ALOR shows that the risk is acceptable

for all eight protozoans, but may be above the ALOR for the two monogeneans (*Dactylogyrus* spp. and *Gyrodactylus* spp.). Thus, additional risk management measures for these two hazards are recommended (Section 12.1).



Photo credit: WorldFish

Genetically improved farmed tilapia (GIFT).

10. Uncertainty of risk estimates

Due to a lack of basic knowledge and the need for precaution, there is always an element of uncertainty in establishing risk estimates for pathogens. In this hazard identification, cells in Table 7 that have a “possible” ranking, particularly for the presence of a hazard in the WorldFish facility, have a high degree of uncertainty. The reason is that this preliminary evaluation does not take into consideration the impact of risk management measures (particularly diagnostic testing) in decreasing uncertainty. A similar ranking for presence of a pathogen in Nigeria is given with a low degree of uncertainty, as the pathogens receiving this ranking, although not reported in the literature for Nigeria, are ubiquitous organisms of wide geographical and host distribution and are highly likely to be present in the country. In Table 8,

which takes to account the risk management measures that WorldFish has either implemented or proposed (Section 7), risk estimates for seven viruses and one fungus are given with a low degree of uncertainty, and all parasites have a moderate level of uncertainty. This latter is due to the complete lack of diagnostic information on the status of WorldFish GIFT stocks with regard to these pathogens. This uncertainty is addressed in Section 12 through the recommendation that GIFT stocks be subjected to parasitological examination. Note that risk for all direct life-cycle parasites, except the monogeneans *Dactylogyrus* and *Gyrodactylus*, is calculated with a low level of uncertainty as being “negligible,” due to these hazards being both ubiquitous globally and treatable in closed aquaculture facilities.



Photo credit: Heba El-Begawi/WorldFish

Hatchery workers harvest Abbassa Nile tilapia from a hatchery in Egypt.

11. Conclusions

Global experience has amply shown that escapes of introduced or transferred species from aquaculture facilities are inevitable. However, good biosecurity should prevent the escape of GIFT from the high-biosecurity quarantine facility to be established in Ogun during the first 2–3 years of the project. During this period of high-biosecurity confinement, the health status of the GIFT can be confirmed before any relocation to other facilities is permitted. In the event that a serious and untreatable disease occurs, the affected subpopulations or entire stock can be destroyed and the facility disinfected.

The risk analysis considered a total of 69 pathogens/pathogen groups as possible hazards for the transfer of GIFT fry from Malaysia to Nigeria. The results show that, should WorldFish and its Nigerian counterparts fully and correctly implement all of WorldFish's in progress and proposed risk management measures as well as the additional measures listed in Section 12, the risk posed by all hazards is likely to fall within the recommended ALOR.

The risk posed by monogeneans belonging to the genera *Gyrodactylus* and *Dactylogyrus* is problematic. This is mainly due to the lack of information as to their possible presence at low prevalence in the WorldFish broodstock, and the large number of species in these genera, which may differ considerably in their pathogenicity to Nile tilapia and some of which may be exotic to Nigeria. It is thus recommended that WorldFish confirm the absence of these parasites through appropriate parasitological examination of GIFT broodstock and fry.

Fish pathogenic bacteria, such as *Aeromonas hydrophila*, *Streptococcus agalactiae* and *S. iniae*, are globally distributed and opportunistic pathogens of freshwater fish. Thus, although these organisms are an important cause of disease and mortality in aquaculture facilities, they are not relevant to this risk analysis. It is also worth noting that currently there are no PCR tests that can differentiate between pathogenic and non-pathogenic strains of these bacteria. This is because pathogenicity is complex, involving several genes, none of which has the properties to be considered a specific virulence factor (Dr. Iddiya Karunasagar, personal communication, 2020).



Tilapia Hatchery in Noakhali, Bangladesh.

12. Recommendations

The following recommendations are made to WorldFish and its Nigerian counterparts to improve the biosecurity management practices that are either in progress or are proposed by WorldFish. They are divided into two categories: (1) those whose implementation is considered essential and (2) those that are desirable.

12.1. Essential actions

- **Monitor implementation of risk management measures.** As the risk assessment is highly dependent upon the risk management measures proposed by WorldFish, monitoring systems should be established to ensure that all risk management measures are fully and effectively implemented.
- **Conduct diagnostic testing for the following additional pathogens.** Viral nervous necrosis virus (VNNV), spring viremia of carp virus (SVCV), tilapia parvovirus (TiPV) and *Aquabirnavirus*. Include the testing as part of the WorldFish protocols, as well as the health certification from the Government of Malaysia that will accompany the shipment of GIFT fry to Nigeria.
- **Ensure the biosecurity of the receiving facility in Nigeria.** WorldFish should ensure that the receiving facility in Nigeria meets all standards for a high-security quarantine facility, as detailed, for example, in Arthur et al. (2007). In preparation for the transfer of GIFT, the receiving facility, which currently houses Nigerian stocks of tilapia and catfish, must have all stocks removed, and all tanks, pipes, surfaces and fomites must then be thoroughly disinfected using an approved method. The holding facility must be upgraded, as necessary, to meet the standards of a high-level biosecurity facility (see Arthur et al. 2007). Specific SOPs for the facility must be developed and in place, and all staff must receive appropriate training to ensure that SOPs are strictly followed.
- **Examine GIFT broodstock for direct life-cycle parasites and treat them, if required.** Many studies have shown that Nile tilapia introduced into new countries many generations ago may still be infected with a wide variety of parasites having direct life cycles. Some of these are host specific to tilapia and thus have clearly been introduced to new environments along with the introduction of their hosts.¹⁴ This risk analysis has determined that a few of these parasites (i.e. *Gyrodactylus*, *Dactylogyrus*) may pose a significant risk to the receiving country, while a number of others, although known or likely to be present in Nigeria, are known to cause disease problems in aquaculture facilities. It is thus recommended that the parent broodstock held in Penang be subjected to full parasitological examination, as it may be possible, through appropriate treatment to minimize the chance that these pathogens will be transferred to Nigeria where they may cause problems in the receiving facility.
- **Develop a detailed contingency plan.** As serious exotic pathogens are known to have escaped from similar high-biosecurity facilities, WorldFish and the GON should develop a detailed contingency plan to deal with such an event, no matter how unlikely. This should include health monitoring of cultured stocks and diagnostic testing to determine the cause of any serious mortality events, as well as planning for efforts to restrict pathogen spread and, where possible, to implement eradication procedures (Arthur et al. 2005).

12.2. Additional recommendations for WorldFish consideration

The following recommendations are not regarded as essential to pathogen risk management but are suggested as useful activities that could be incorporated into project design:

- **Study the diseases of fish species near GIFT aquaculture facilities.** To better understand the potential for pathogen transfer between wild and cultured stocks, baseline studies of the diseases of fish species in the vicinity of GIFT aquaculture facilities in Nigeria should be conducted. Such monitoring will also help detect any transfer of introduced exotic pathogens from GIFT to wild finfish populations.
- **Commission an expert review of testing protocols.** WorldFish may wish to have an independent expert in fish disease diagnostics review the proposed testing protocols for the final list of pathogens used to certify GIFT fry.
- **Stress test GIFT to reveal hidden pathogens.** Stress testing of GIFT broodstock and fry could be conducted to check for cryptic or unknown pathogens.
- **Organize a collaborative study of Nigerian fish pathogens.** As the pathogens of cultured fish of Nigeria remain poorly known, WorldFish should consider developing an international project to conduct baseline surveys of key cultured species. Such studies should include viruses, bacteria and parasites.
- **Prepare a fish parasite checklist.** As there is no comprehensive listing or critical review of the parasites reported from Nigerian fish, a starting point would be the preparation of a checklist of the parasites of Nigerian fish. The preliminary list of publications on the diseases of Nigerian fish given in Annex 1 could serve as a starting point for this work.
- **Assist in a decision on Nigeria's ALOP.** WorldFish should initiate discussions with the GON and relevant stakeholders with the aim of determining the national ALOP. A national consultation involving the plant, terrestrial animal and aquatic animal health sectors could be convened to assist in reaching a consensus among stakeholders.
- **Develop a national strategy for aquatic animal health.** WorldFish could assist the GON in developing a national strategy for aquatic animal health and in participation in the FAO's PMP/AB program.
- **Implement measures to prevent future transfers of tilapia.** The GON should take all possible measures to prevent the future transfer of tilapia of unknown health or genetic status from outside the national territory.



Photo credit: Balaam Mehalder/WorldFish

Tilapia brooding female.

Notes

- ¹ Box 1216, Barriere, B.C., Canada, V0E 1E0; e-mail: jraconsulting@xplornet.ca
- ² See, for example, Briggs et al. 2004; Bondad-Reantaso and Subasinghe 2005; Bondad-Reantaso et al. 2005a and 2005b; Flegel 2006; Johnston 2008; Rodgers et al. 2011; Tavares-Dias and Martins 2017; Shinn et al. 2018.
- ³ ICES (2005) defines a “transferred species” as “Any species intentionally or accidentally transported and released within areas of established populations, and continuing genetic flow where it occurs,” while an “introduced species” is defined as “Any species transported intentionally or accidentally by a human-mediated vector into aquatic habitats outside its native range.”
- ⁴ <https://naqsportal.net/>
- ⁵ See, for example, Section 4 in Arthur et al. 2008.
- ⁶ The nomenclature for fish used in this document follows Froese and Pauly 2019.
- ⁷ See, for example, Dong et al. 2017b.
- ⁸ See, for example, DAWE 2020.
- ⁹ For additional information, see the review of Ibrahim 2020.
- ¹⁰ See, for example, The Fish Site 2021.
- ¹¹ See, for example, Arthur and Lumanlan-Mayo 1997.
- ¹² See, for example, Arthur and Lumanlan-Mayo 1997.
- ¹³ The OIE’s Aquatic Animal Health Code (2019a) defines entry assessment as consisting of describing the biological pathway(s) necessary for an importation activity to introduce a pathogen into a particular environment, as well as estimating the probability of that complete process occurring, either qualitatively (in words) or quantitatively (as a numerical estimate). An exposure assessment is defined as consisting of describing the biological pathway(s) necessary for exposure of animals and humans in the importing country to the hazards (in this case the pathogenic agents) from a given risk source, and estimating the probability of these exposure(s) occurring, either qualitatively (in words) or quantitatively (as a numerical estimate). Most countries consider that the “entry” pathways terminate and the “exposure” pathways begin at the importing country’s border, a practice that is followed in this risk analysis.
- ¹⁴ See, for example, Natividad et al. 1986.

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Author's qualifications

J. Richard Arthur, MSc, PhD

Dr. J. Richard Arthur has more than 45 years of experience in aquatic animal health. He received his MSc (parasitology) and PhD (fisheries parasitology) from the University of Calgary and completed a US Academy of Sciences Scientific Exchange Visit to the Soviet Union and Czechoslovakia, as well as a Department of Fisheries and Oceans post-doctoral fellowship and an industrial research fellowship for the Natural Sciences and Engineering Research Council in Canada before serving as a project advisor to the Bureau of Fisheries and Aquatic Resources in Manila, Philippines, and the International Development Research Centre's fish health network coordinator. After serving briefly as the International Development Research Centre's program officer (fisheries and aquaculture) based in Singapore, he returned to Canada in 1989 as head of the parasitology section of DFO's Maurice Lamontagne Institute, where he conducted research on the taxonomy of fish parasites and their use as biological tags for stocks of commercially important fish.

Since 1997, Dr. Arthur has been an independent international consultant on aquatic animal health based in Barriere, British Columbia. He has working experience in more than 50 countries through contracts with FAO, the Network of Aquaculture Centres in Asia-Pacific, the Secretariat of the Pacific Community, the Asian Development Bank and the private sector. His consultancy work has focused mainly on risk analysis for aquaculture, biosecurity and national aquatic animal health policy development and strategic planning.

He is the author or editor of more than 100 scientific publications, including several volumes on the application of risk analysis to the aquaculture sector.



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Annex 2

Outline of a risk management plan

Preshipment activities

A. By WorldFish, Penang (pre-border activities)

- Successful completion of first diagnostic testing of G17 Broodstock (completed).
- Successful completion of second diagnostic testing of G17 Broodstock.
- Review the expert risk analysis.
- Review/revision of the pathogen list.
- Successful completion of diagnostic testing of G17–F1 swim-up fry.
- Examination of G17 broodstock for direct life-cycle parasites (recommended).
- Successful certification by Malaysia Competent Authority of fry to be shipped.

B. By Nigerian counterparts, in cooperation with WorldFish

- Establishment of a high-biosecurity quarantine facility (HSQF) in Ogun.
- Development of HSQF SOPs for arrival and transfer of fry, destruction or sanitary disposal of shipping containers, shipping water and any mortalities, etc.
- Training of HSQF staff.
- Establishment of an independent national scientific advisory team, including drafting TOR.
- Development of a monitoring and diagnostic testing program for G17–F1 to F3.
- Development of a monitoring program for wild cichlid populations.
- Development of a detailed contingency plan, including designated emergency funds.

Border activities

- Clearance of GIFT fry shipment by the GON.

Post-border activities

- Secure transfer of GIFT fry to the Ogun facility.
- Sanitary disposal of transportation water and packaging.
- Monitoring of health status of G17–F1 to F3.
- Conducting of diagnostic testing at prescribed intervals.
- Development of SOPs and other standards for NBCs.

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.

WorldFish is part of One CGIAR, the world's largest agricultural innovation network.