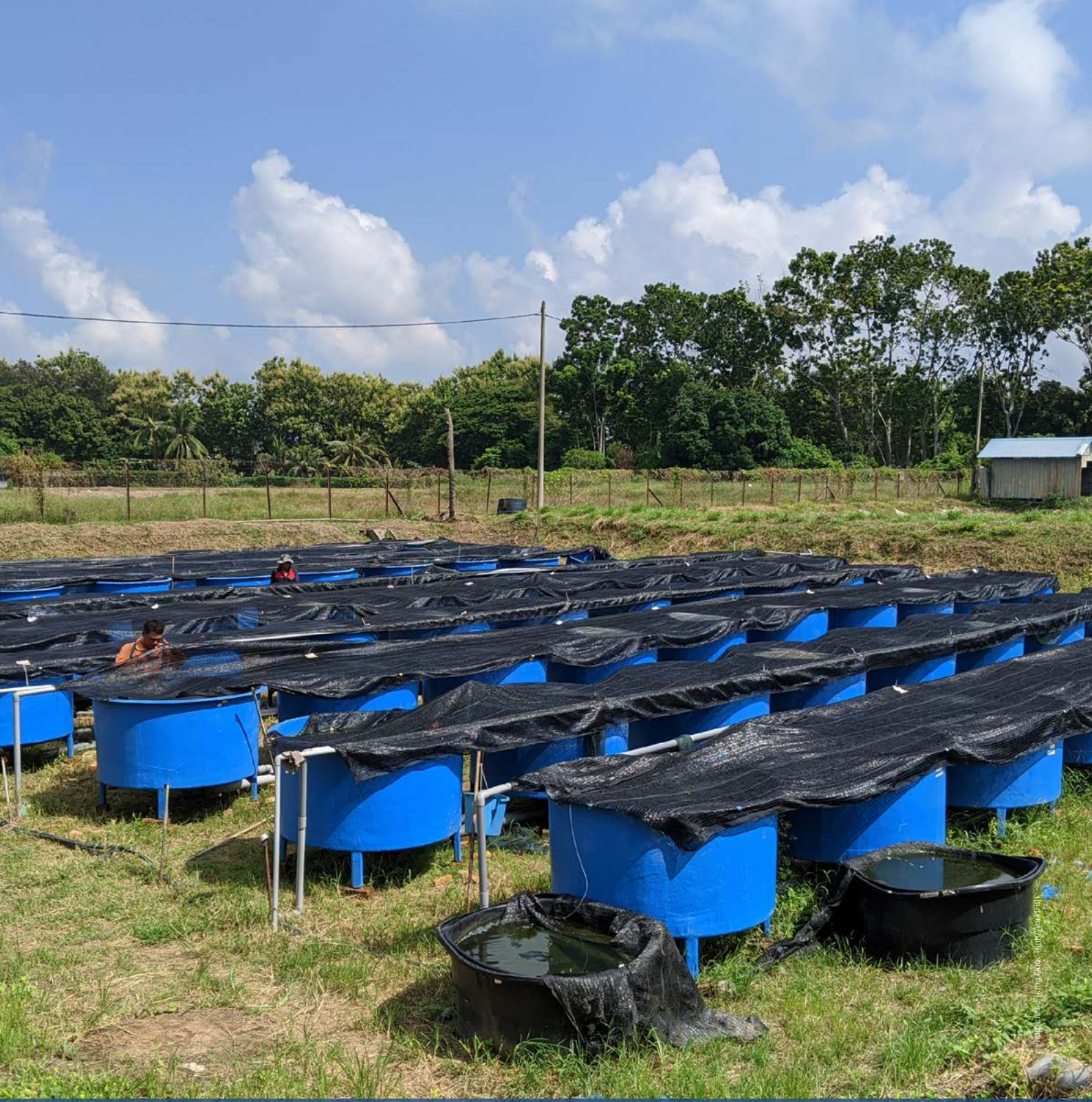

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GIFT transfer risk management: Genetics

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GIFT transfer risk management: Genetics

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Executive summary

In summary, the risk of direct genetic impacts on Nigerian populations of Nile tilapia are impossible to assess due to a lack of relevant research. The risk of direct genetic impacts on other tilapia populations is low, and the risk of indirect genetic impacts on all but one tilapia species in Nigeria is also low.

This report is a genetic risk analysis of introducing Genetically Improved Farmed Tilapia (GIFT) in Nigeria for aquaculture purposes. It was conducted following the guidance of the International Council for the Exploration of the Sea's Code of Practice for the Introductions and Transfers of Marine Organisms (ICES Code) as well as the Food and Agriculture Organization's (FAO) "Precautionary approach to capture fisheries and species introductions" (1996).

GIFT is a strain of Nile tilapia (*Oreochromis niloticus*) that has been genetically improved for about 17 generations. Although derived from Nile tilapia, the GIFT genotype would not normally be found in natural populations of the species. Nile tilapia has been identified as a potential invasive species in several countries and areas of the world, such as Australia, Lake Victoria, Philippines, Madagascar, Nicaragua and the Zambezi Basin.[1]

Nile tilapia is native to Nigeria. Strains of it, including GIFT, have been introduced into Nigerian aquaculture and are currently being farmed in the country.

Nile tilapia and GIFT have escaped and formed feral populations or contributed genes to tilapia populations in Africa, Asia and Latin America. For the purpose of this analysis, it is assumed that feral populations of GIFT will become established in waterbodies that contain Nile tilapia and other tilapia species, and that hybridization with some of these species is possible. As a result, changes in the genotype, and therefore fitness, of several native tilapia species in Nigeria is possible.

A literature search revealed that GIFT genes have been found in natural populations of Nile tilapia, but no examples were found where hybridization between GIFT and its wild relative, Nile tilapia, resulted in adverse genetic impacts on natural populations. However, no detailed studies on the genetic impact of this hybridization have been conducted. So although possible, the risk of direct genetic impacts is difficult to determine.

In light of the fact that GIFT is a strain of Nile tilapia, it could be assumed to perform similarly once established in Nigerian waters. Nile tilapia has been shown to have adverse impacts on *O. mossambicus* and other *Oreochromis* species in other countries. However, it is difficult to partition the adverse impacts between ecological and genetic causes.

The other species that could be impacted by direct genetic effects of hybridization, meaning changes in genotype, include the mouth brooding species of tilapia: *O. aureus, Sarotherodon melanotheron* and *S. galilaeus*. Natural hybridization between GIFT and other mouth brooding tilapia is uncommon in nature, and the examples of hybridization come from artificial breeding. So although possible, the risk of direct genetic impacts is low.

Tilapia species that could be impacted by indirect genetic effects include the above mouth brooding species as well as substrate spawners *Coptodon guineensis, C. dageti, C. zillii* and *Pelmatolapia mariae*.

There is an absence of direct evidence that changes in the genotype of the tilapia species in Nigeria have resulted in functional changes in the phenotype or in changes in the fitness of the species. In light of this, it is impossible to predict the level of harm resulting from their hybridization with GIFT. However, given the low probability of feral GIFT hybridizing with Nigerian tilapia other than Nile tilapia, the risk of adverse genetic impacts is low.

Indirect genetic impacts between GIFT and Nigerian tilapia populations are possible through ecological interactions, such as predation and competition. These would reduce the size of native tilapia populations to the point that inbreeding and loss of important genetic diversity reduce their long-term survivability. In such cases, it is often difficult to determine whether it is the ecological interaction or loss of genetic diversity per se that is the specific cause of harm. Critical experiments to address the difficulty have not been conducted.

Evidence of genetic harm from direct and indirect effects of GIFT on other tilapia populations is lacking. However, there is evidence from other species that the genetic resources of wild populations of aquatic species have value and should be conserved. The natural genetic differences among the founding strains and stocks that were used to establish the GIFT strain were responsible for the excellent performance of GIFT in a range of aquaculture settings. The behavior of tilapia species and the genetic distance of Nigerian populations from the rest of Africa indicate that local genetic differences and adaptations could represent important genetic resources for aquaculture and the long-term fitness of Nigerian populations.

Given the lack of information on the harm caused by both direct and indirect genetic effects on native tilapia populations in Nigeria, the ICES Code and FAO's precautionary approach are recommended as a risk management strategy. The ICES Code and the proposed protocol for WorldFish GIFT transfer to Nigeria call for establishing an independent advisory body to oversee the introduction of GIFT. This advisory body should remain active and monitor the results.

The precautionary approach calls for establishing target and limit reference points, pre-agreed actions when reference points are reached or breached, and a monitoring system. This approach allows development to continue in the face of uncertainty and is recommended here to include the following:

- genetically categorizing GIFT transferred from Malaysia
- genetically categorizing the native tilapia populations with which GIFT may interbreed
- establishing target and limit reference points for key genetic and training parameters
- monitoring the genetic resources of aquaculture facilities using GIFT to ensure proper genetic resource management
- monitoring surrounding waterbodies for the presence of GIFT or GIFT markers
- establishing conservation areas where important native tilapia genetic resources should be protected and where farming GIFT should be excluded
- training and awareness raising on the management and importance of genetic resources.

Introducing GIFT into Nigeria under the auspices of WorldFish presents an excellent opportunity to conduct critical research on the direct genetic impacts of GIFT on native tilapia species. WorldFish is advised to include such research in its program that could fill a gap in the scientific literature on how changes in genotype are reflected in changes in phenotype, fitness and the long-term survival of native tilapia species in Nigeria.

Assumptions and terms of reference

The following are the tasks laid out in the genetic risk management plan:

- Review the information provided by WorldFish on genetic aspects associated with the proposed transfer.
- Conduct, with the assistance of WorldFish (if requested), a detailed review of the relevant literature dealing with the genetic impacts of previous transfers of Nile tilapia.
- Follow current best practices, which may include
    - the general methods outlined in the FAO Fisheries and Aquaculture Technical Paper No. 519
    - the guidelines given in the ICES Code
    - the procedures outlined in Annex B: Risk Review of the ICES Code (ICES 2012, Appendix B: Risk Review, pp. 256–262).
- Assess both direct and indirect genetic risks to the receiving environment that may result from the proposed transfer.
- Supply a document that provides an assessment of the genetic risks associated with the proposed transfer and outlines a recommended genetic risk management plan, including risk management measures, that could be implemented before, during and after transferring GIFT from Malaysia to Nigeria.

Taxonomic note

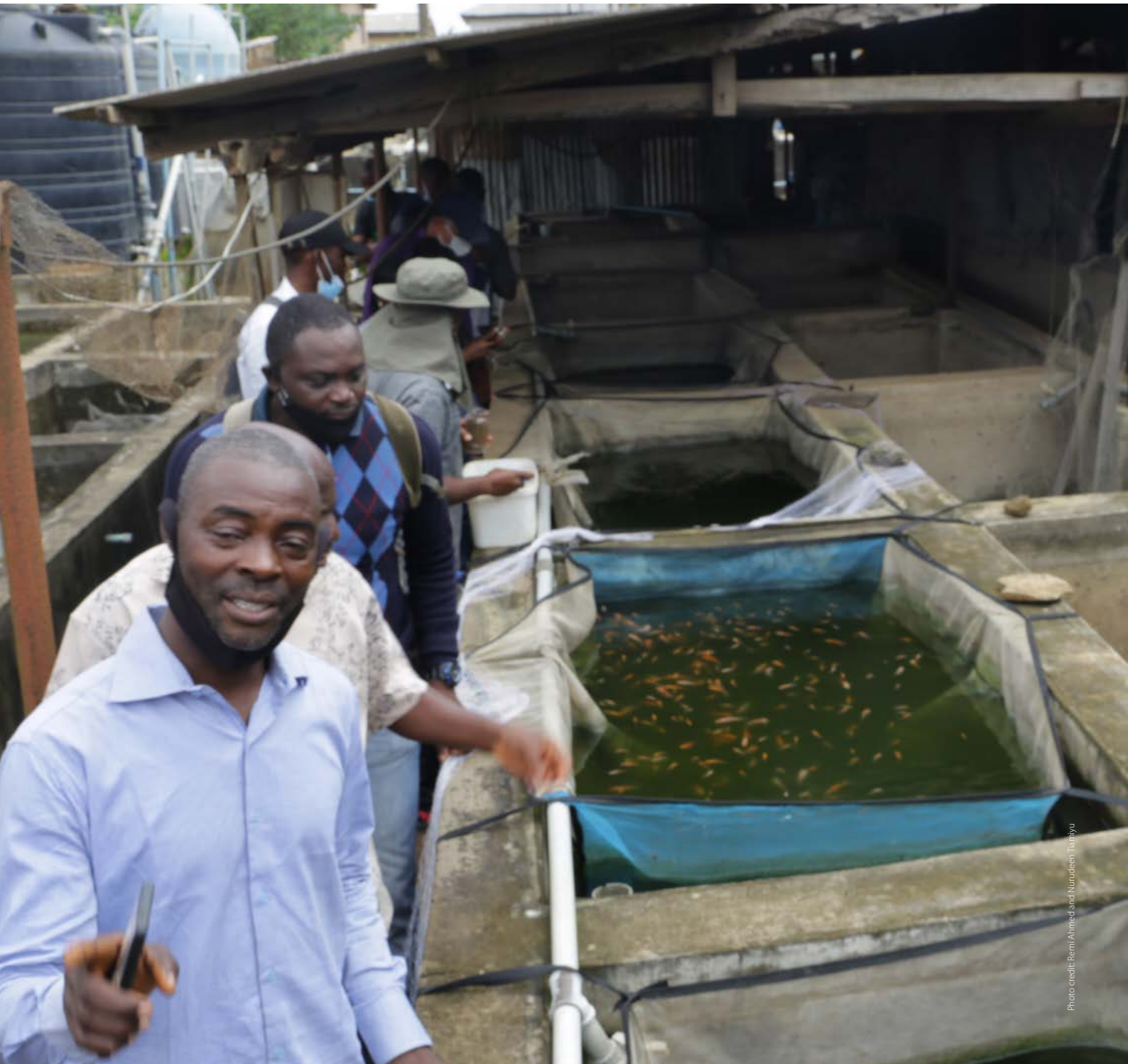
The names of *Tilapia dageti, T. guineensis, T. zillii* and *T. mariae* have been reclassified as *Coptodon dageti, C. guineensis, C. zillii,* and *P. mariae*, respectively (Dunz and Schliewen 2013). Past publications may refer to the older invalid names, but the updated names are used in this document.

Timeframe

- The duration of the project was from December 15, 2020, to February 15, 2021.
- Perform the work as outlined above during the stipulated time.
- Submit first draft to WorldFish by January 15, 2021.
- Submit WorldFish comments to the expert panel by January 30, 2021.
- Submit the final draft to WorldFish by February 15, 2021.

1. Introduction

As stated in the terms of reference (TOR) for this project, risk analysis involving the introduction of GIFT to Nigeria for aquaculture purposes follows the guidance in the ICES Code and other FAO documents. As outlined in the ICES Code and by FAO, risk analysis in this project includes the direct and indirect impacts of GIFT on genetic resources of native species in Nigeria. The assessment of those risks and possible management strategies are presented in section 2.



Red tilapia fry in hapas, Latia Global Investment Limited, Lagos, Nigeria.

2. Review of the genetic impact of previous introductions of GIFT and Nile tilapia

2.1. Hazard identification: Genetic impact of GIFT

GIFT is a strain of Nile tilapia. Nile tilapia has been referred to as "invasive" and capable of causing harm, specifically adverse ecological and genetic impacts, in numerous scientific publications (Canonico et al. 2005; Eknath and Hulata 2009; Blackwell et al. 2020). Hallerman (2008) provided a useful framework for identifying genetic hazard and harm from the introduction of non-native species:

"A harm is defined as gene pool perturbation resulting in negative impacts to a species, a hazard is an agent or process that has the potential to produce harm."

The hazardous agent in this case is a new, feral stock of GIFT that has the potential to be present in Nigeria's water bodies. The direct genetic harm of concern is the movement of genes from the feral stock into wild populations of Nile tilapia or into other species with which GIFT may hybridize or introgress. Hybridization or introgression can create potentially harmful changes in the genetic adaptation of local populations of the same or related species and a loss of long-term fitness. There are a number of mechanisms by which this loss of local adaptation and fitness might occur. These include genetic swamping leading to reduced genetic variance and loss of adaptability, outbreeding depression, etc.

GIFT was developed by WorldFish (formerly the International Center for Living Aquatic Resources Management) in the 1980s from eight different strains and stocks of Nile tilapia collected from various parts of Africa (Eknath et al. 1993). So although GIFT is a Nile tilapia, it contains genes from a variety of locations; the GIFT genotype would not normally be found in nature. A recent genetic study of tilapia in Ghana, including GIFT, revealed the presence of *O. mossambicus* genes in GIFT (Anane-Taabeah et al. 2019), possibly arising from one source population of GIFT being hybridized. The GIFT genotype has been proven to be an excellent genotype for aquaculture purposes. GIFT or GIFT-derived strains now account for a significant portion of tilapia production in Asia (Bentsen et al. 2012 and 2017) and are expected to make major contributions to African aquaculture (Anane-Taabeah et al. 2019; WorldFish n.d.).

The GIFT breeding program strives for robustness, specifically in producing a good fish in many environments (Agha et al. 2018). However, it has recently been shown to display genotype by environment interaction (GxE) (Agha et al. 2018). That is, the performance or impact of the fish may be different in different environments. For example, GIFT escaping from a freshwater aquaculture facility into a brackish water environment would not be expected to become established or invasive, whereas the same fish (and genotype) escaping into a freshwater environment would be expected to survive. However, the magnitude of the GxE interactions as they relate to invasiveness, specifically harm, throughout the range of GIFT rearing sites has not been examined.

WorldFish currently maintains the GIFT strain in its breeding facilities in Malaysia, for distribution as appropriate. The organization keeps rigorous breeding records and practices genetic resource management to ensure that the GIFT strain maintains appropriate genetic diversity and avoids inbreeding. Therefore, GIFT continues to be an excellent genotype for aquaculture uses (Bentsen et al. 2012 and 2017; Ponzoni et al. 2010). However, the genetic impact of GIFT on wild relatives and related species has not been well evaluated.

2.2. Distribution of Nile tilapia and GIFT

The original natural distribution of the genetic resources of Nile tilapia extended from 8^0N to 32^0N. This area covered the Nile River and its tributaries and lake systems in the north, the watersheds of the lakes of Tanganyika in the south (Trewavas 1983) and the sub-Sahelian watersheds

of West Africa, including the basins of rivers Niger, Volta, Gambia and Senegal (Teugels et al. 2003). According to FishBase,[2] Nile tilapia naturally occurs in coastal rivers of Israel, the Nile basin (including lakes Albert, Edward and Tana), Jebel Marra, Lake Kivu, Lake Tanganyika, Awash River, various Ethiopian lakes, the Omo River system, Lake Turkana, Suguta River and Lake Baringo. In West Africa, natural distribution covers the basins of Senegal, Gambia, Volta, Niger, Benue and Chad, with introduced specimens reported from various coastal basins. As such, natural stocks of Nile tilapia exist in Nigeria with which the introduced GIFT could interbreed.

Lind et al. (2019) reported that eastern and western populations of Nile tilapia are genetically different and that there are three broad genetic groupings of the species: (i) Sudano-Sahelian populations, covering West Africa, including Nigeria, (ii) Great Rift Valley populations in Ethiopia and (iii) Nile drainage and Great Rift Valley populations in Kenya. The pattern of genetic differentiation in Sudano-Sahelian populations is best explained by the "isolation by distance model" and by major river systems (Lind et al. 2019). The Sudano-Sahelian group has been shown to possess more genetic diversity than previously thought and at a more fine-scale degree of differentiation than predicted by major river systems. This may be due to the social and breeding behaviors of Nile tilapia that would allow local populations to become genetically different from other nearby populations.[3]

GIFT has been widely disseminated outside Malaysia and the research areas of the Philippines where it originated. Officially, it has been used in 11 countries for experimental or dissemination purposes: Bangladesh, Brazil, India, Indonesia, Fiji, Malaysia, Myanmar, Philippines, Thailand, Timor-Leste and Vietnam. There are also records of unofficial transfer of GIFT to other African countries, including Nigeria (Anane-Taabeah 2019; WorldFish n.d.). According to Nigeria's official country report to FAO (2016), Nile tilapia is currently farmed in Nigeria and has undergone selective breeding, monosex (all male) production, chromosome manipulation and hybridization. Nigeria's country report specifically states that GIFT has been farmed in the country. Nile tilapia has also been introduced from other African nations (FAO 2016). Thus, non-native genotypes of Nile tilapia including GIFT have been and are currently used in Nigerian aquaculture.

Studies on the genetic resources of tilapia in Nigeria in relation to West Africa are scarce, so it is difficult to determine the degree of difference between the genetic resources of Nigeria and other West African countries. Ukenye et al. (2019) found low but comparable levels of genetic variation in *C. guineensis* in Nigeria as compared to other West African countries. Differences would imply that the genetic resources in Nigerian tilapia would be especially valuable or represent a significant evolutionary unit.

Genetic analyses of Nile tilapia and GIFT indicate that there are sufficient genetic markers or polymorphisms that can help differentiate GIFT from other strains of Nile tilapia and track GIFT's potential hybridization with other tilapias in nature (Peñaloza et al. 2021). Van Bers et al. (2012) stated, "We expect that this set [of polymorphisms] will be widely applicable for use in tilapia aquaculture, e.g. for pedigree reconstruction. In addition, this set is currently used for assaying the genetic diversity of native Nile tilapia in areas where tilapia is, or will be, introduced in aquaculture projects. This allows the tracing of escapees from aquaculture and the monitoring of effects of introgression and hybridization."

2.3. Additional tilapia species in Nigeria

There are several other species that are (or were) called "tilapia" that naturally occur in Nigeria. GIFT has the potential for both direct and indirect genetic impacts with the species listed in Table 1.

Species	Distribution and spawning habit	Potential to hybridize with GIFT	Conservation status
Coptodon guineensis	Coastal streams even full seawater in Nigeria; substrate spawner	Negligible	Least concern
C. dageti	Hadejia-Nguru wetlands and lakes of the middle Niger system; substrate spawner	Negligible	Possible endemic (Abubakar et al. 2015) but Least concern in FishBase
C. zillii	Highly adaptable and tolerates varying water qualities, a wide range of temperatures and salinities; substrate spawner	Negligible	Not evaluated
Oreochromis aureus	Freshwaters and brackish waters; mouth brooder	Possible	Not evaluated (possible invasive)
Pelmatolapia mariae	Coastal lagoons and lower rivers; substrate spawner	Negligible	Least concern
Sarotherodon melanotheron	Lagoons and estuaries; mouth brooder	Possible	Not evaluated (possible invasive)
S. galilaeus	Lakes and rivers; mouth brooder	Possible	Near threatened

Source: FishBase 2021 (except as noted).

Table 1. Species of "tilapia" naturally occurring in Nigeria.

3. Risk assessment of genetic impacts

As defined by Hallerman (2008), risk is "the product of the probability of exposure, P(E), and the conditional probability of harm given that exposure has occurred, P(H|E). That is, R = P(E) x P(H|E)." It is assumed that GIFT will escape from breeding and grow-out facilities in Nigeria, so the probability of exposure is 1. The probability of harm P(H/E) is the important variable in determining the degree of risk for this project. The risks of direct and indirect impacts are evaluated in section 3.1.

3.1. Direct impacts

3.1.1. Hybridization within native *O. niloticus*

In light of the fact that GIFT is derived from Nile tilapia, hybridization is possible and probable if the two groups meet in nature. Farmed Nile tilapia, including GIFT, have escaped and have bred with native stocks in Ghana (Anane-Taabeah et al. 2019). Does this breeding create harm? Non-native salmonid genotypes have been recognized as a potential harm to native salmonid genotypes (Fleming et al. 2000) through outbreeding depression and disruption of co-adapted gene complexes. However, most of the evidence of outbreeding has come from studies on salmonids and salmonid stocking programs (Araki 2007). Tibihika et al. (2020) mentioned that mixing different stocks/strains of Nile tilapia could possibly lead to outbreeding depression, but no evidence was given. Similarly, Anane-Taabeah et al. (2019) documented very well that aquaculture is a vector for spreading non-native genes to native tilapia populations, but there was no assessment of the actual biological or phenotypic impact on native Nile tilapia—meaning no assessment of harm. A search of Scopus, Elsevier's abstract and citation database, specifically for outbreeding depression between mating of different strains/stocks of Nile tilapia resulted in no references being found. This is in contrast to the adverse impacts found from Nile tilapia hybridizing with other species of tilapia (section 3.1.2) in areas outside of Nigeria.

It is possible and probable that genetic differences exist within Nile tilapia in Nigeria. However, research has not been conducted to reveal whether these differences are associated with specific fitness traits or traits that are of specific interest to aquaculture. It is probable that the genotype of hybridized populations of GIFT and Nile tilapia will change, but how that change will impact fitness and survivability of the populations is not known.

However, it is likely that populations of native Nile tilapia would have genetic resources of potential value, as was found in developing the GIFT strain in the first place and as has been found in other farmed species (Eknath et al. 1993). That is, unique genetic resources appear to be of value in the future, even if their present use is not known. Additionally, distinct genetic resources may indicate an evolutionary significant unit (ESU) (Moritz 1994). According to Moritz (1994), "the term 'significant' in ESU should be seen as a recognition that the set of populations has been historically isolated and, accordingly, is likely to have a distinct potential."

GIFT is a domesticated strain of Nile tilapia that possesses characteristics that are beneficial to aquaculture but may not be in the wild, such as surface feeding on pelletized food and lack of predator avoidance.[4] The genetic basis for these traits, however, is not well known. These characteristics may reduce the fitness of GIFT in the wild and the chance of adverse impacts on native stocks, though escaped domesticated rainbow trout were shown to be able to survive in the wild (Araki et al. 2007). It must be assumed that GIFT would escape and interbreed with native stocks of Nile tilapia. However, there is little direct evidence that they would have specific adverse impacts on native gene pools of Nile tilapia in Nigeria other than changing their genotype. The resulting changes in phenotype and fitness, specifically harm, are not possible to determine at this time.

3.1.2. Hybridization with other tilapia species

Nile tilapia and other species of *Oreochromis* can hybridize, and there are four broad categories of result: (1) no further interbreeding after the initial hybridization (F1 generation), (2) a mixture of introgressed and pure populations, (3) complete admixture of the two species,

meaning a hybrid swarm, and (4) total displacement of one species. Hybrid tilapia can display poor growth, lower survival in the wild and deformities (Eknath and Hulata 2009).

Nile tilapia, including GIFT and all members of the genus *Oreochromis*, are maternal mouth brooders, so hybridization with substrate spawners, such as those from the genera *Captodon*, under natural conditions is not possible. Nile tilapia has been shown to hybridize with other mouth brooding tilapias. It has been shown to hybridize with *S. galilaeus* in cages (Otubusin and Olu 1988), with progeny of the cross capable of superior growth rate. Akian et al. (2017) has documented hybridization between Nile tilapia and *S. melanotheron* in aquariums. Natural hybridization in nature has not been observed.

Nile tilapia and *O. aureus* share common distribution and yet do not readily hybridize in nature, though they can be hybridized in aquaculture facilities.[5] Nile tilapia introduced into Lake Victoria was a leading factor in the near extinction of the endemic *O. esculentus*.[6] Nile tilapia also displaced *O. macrochir* in a lake in Madagascar (Eknath and Hulata 2009), though both of these species were introduced to the island. De Silva et al. (2004) concluded that explicit scientific evidence is lacking to demonstrate that any of the tilapia introductions into the Asia-Pacific region have actually impacted natural levels of biodiversity. There is evidence that Nile tilapia has impacted biodiversity in Latin America (McCrary et al. 2007), but it is unclear whether the mechanism for the displacement was genetic or ecological.

Firmat et al. (2013) noted that Nile tilapia can be considered invasive and has interbred with *O. mossambicus* in the Limpopo River system with the result being a mixture of introgressed populations and pure populations of *O. mossambicus*. The failure of Nile tilapia to hybridize throughout the Limpopo system was thought to depend on the distance from original introduction, a variable ecological environment and existence of several refugia for *O. mossambicus* in the system.

However, as Blackwell et al. (2020) point out, "the full evolutionary and ecological consequences of hybridization between invasive and native species are typically unclear, and further studies of the impact of hybridization events on native biodiversity are required." Studies on the tilapia species in Nigeria have not been conducted to determine if the native species of Nile tilapia is hybridizing with other mouth brooding tilapia species.

3.2. Indirect genetic impacts

Indirect genetic impacts, meaning those where GIFT does not interbreed with local populations, arise from two different, but related, pathways:

1. from ecological interactions between the escaped GIFT and local populations
2. from increased "invasiveness" of the escaped GIFT or of GIFT hybrids that further increases ecological interactions.

Both of these pathways have the potential to reduce the effective population size of local populations. The resulting impacts are a loss of adaptive genetic variation, loss of genetic resources that may have future value, and increased inbreeding depression.

3.2.1. Hybridization within *O. niloticus*

For the purpose of this risk analysis, it is assumed that GIFT will interbreed with local populations of Nile tilapia. Therefore, by definition, there are no indirect genetic impacts.

3.2.2. Hybridization with other species

As stated in section 3.1.2, GIFT may or may not hybridize with other tilapia species under certain conditions, so indirect genetic impacts are possible. Hallerman (2008) stated that, "indirect genetic harm results because competition or predation reduces the abundance of affected populations leading to loss of genetic variability and ability to adapt in face of changing selective pressure, and an increased likelihood of subsequent inbreeding and extinction." The indirect result of an increase in invasiveness of GIFT or GIFT x local hybrid would be increased competition, predation and interference with reproduction. According to J.R. Arthur (Personal Communication, 2012), "Extinction could happen relatively quickly as a purely ecological effect, or slowly if the population size of an affected species is reduced to a level where inbreeding and random genetic drift drive it into a negative fitness loop (an "extinction vortex").

The genetic resources of tilapia species in Nigeria have not been well studied, so it is difficult to determine the impacts from loss of genetic variation, increased inbreeding and reduced effective population size. However, most of the species populations in Table 1 seem viable, and there appears to be little concern for their conservation status. Those listed have large ranges and wide environmental tolerances, and several are even listed as potentially invasive. However, the International Union for Conservation of Nature (IUCN) listed *S. galilaeus* as "near threatened" in 2007, and special attention should be paid to mitigating and monitoring indirect genetic impacts on this species.

Hallerman (2008) stated that regarding indirect genetic impacts, "Exact probabilities of risk are difficult or impossible to determine for all types of possible harm. Indeed, it is unlikely that all possible harms would be known a priori, particularly with respect to any indirect effects" and that "it will be necessary to update the risk analysis as knowledge accumulates using an adaptive management approach." Given the distribution and population status of tilapia species in Nigeria, the risk of adverse indirect genetic effects is considered low, except for *S. galilaeus*.

Photo credit: Remi Ahmed and Nurudeen Tiamiyu

Tilapia in floating cages (Nigeria).

4. Management and mitigation of genetic risks

As stated in the TOR, this risk analysis follows the recommendations of, among other things, the ICES Code and other FAO documents. The ICES Code is an important framework for assessing the introduction of aquatic species to areas outside their native range. The inland regional fisheries bodies of FAO have, in principle, adopted the code, so it applies to areas beyond the marine environment.[7] The ICES Code addresses, among other things, the evaluation of potential genetic risks associated with the transfer of aquatic organisms. As a result, conformation with the recommendations of the code can be considered a best practice when introducing new species into a country for aquaculture development and is recommended here.

The ICES Code identifies three broad areas of activity:

1. recommended procedure for all species prior to reaching a decision regarding new introductions
2. if the decision is taken to proceed with the introduction
3. recommended procedure for introduced or transferred species that are part of current commercial practice.

The code also calls for the submission of an import proposal (transfer proposal) and the establishment of an impartial and independent group of experts to review the proposal to import a species and provide advice. ICES further has a dedicated working group on species introductions that not only helps review proposals but reviews the ongoing status of species introductions.

Another key guiding principle to reduce the chance of harm from introduced species is the precautionary approach as defined by FAO (1996). This approach calls for, among other things, the following:

- establishing target and limit reference points
- agreeing on pre-agreed actions when reference points are reached
- monitoring to track the trajectory toward the reference points.

The precautionary approach is a means to deal with uncertainty and proceed with development in the absence of complete information. The establishment of reference points and pre-agreed actions needs to involve key stakeholders and sectors, such as private industry, government, academic institutions and conservation. Applying the approach and involving key sectors widely were effective in bringing groups together on the issue of tilapia introduction in Cape Verde (Bartley et al. 2012) and would be expected to function similarly in Nigeria.

4.1. Recommended procedure for all species before reaching a decision regarding new introductions

The main action recommended by ICES prior to an introduction is the development of a comprehensive proposal that outlines, among other things, the species, its native range, the donor population, the life history stage to be introduced, its genetic character and the intended use and location of the introduced species. WorldFish's proposal (n.d.) includes such a recommendation and should be continuously modified based on the inputs of this and other risk analyses by an independent advisory panel.

WorldFish aquaculturists and resource managers from the Nigerian government should confirm the genetic character of the transferred yolk sac fry before transfer to Nigeria using the suite of polymorphisms mentioned in section 2.2. Additionally, WorldFish should provide a list of useful genetic markers and/or a suite of polymorphisms (for example, those from Peñaloza et al. 2021 and Van Bers et al. 2012) that will uniquely identify the transferred strain to help with future monitoring and impact assessment. If such a list of markers or useful polymorphisms does not exist to trace sufficiently the spread of GIFT in Nigeria, then WorldFish should help develop them through collaborative research and seek appropriate funding.

4.2. If the decision is taken to proceed with the introduction

As outlined in the transfer proposal (WorldFish n.d.) introduced GIFT should be used to establish a broodstock for the production of progeny for further grow-out. According to the transfer proposal, transferred yolk sac fry will be kept in a designated land-based, secured quarantine facility in Nigeria's Ogun State. They will be raised with regular health checks. Progeny resulting from the originally transferred stock will be transferred to Delta State for breeding and for grow-out. The regular health checks should be supplemented with occasional assessment of genetic resources to ensure genetic diversity is not being lost in subsequent generations.

A strategic monitoring program should be established in key areas where GIFT may enter Nigerian waters. Best aquaculture practices dictate strong measures to prevent farmed fish from escaping. However, it is assumed that GIFT will escape or be illegally transferred to areas not authorized for their use, so monitoring the surrounding environment for GIFT will be essential. Potential GxE interactions could yield different levels of invasiveness, meaning harm, from GIFT that have been established in different water bodies, so several important locations would need monitoring (Agha et al. 2018). Monitoring could involve genetic analysis of the markers unique to GIFT and the set of polymorphisms that help distinguish GIFT, as well as visual examination of tilapia. An annual progress report should be submitted to the independent national advisory team for review.

Capacity building and awareness raising activities for key stakeholders will be necessary to reduce the risk of illegal transfer of GIFT and to increase awareness of GIFT in Nigeria. To ensure the continued good genetic resource management of GIFT as practiced by WorldFish (Bentsen et al. 2017), capacity building in the breeding facilities in Ogun and Delta states will be necessary.

WorldFish, in collaboration with Nigeria's Department of Fisheries and Aquaculture, should set up an independent national advisory team consisting of representatives from key stakeholder groups and wider civil society. The national competent authority (Department of Fisheries and Aquaculture) should lead the group meetings and dialogue toward building consensus on national implementation of the risk management plan and recommendations. This group would be similar to the ICES Working Group on Introductions and Transfers of Marine Organisms and could help establish reference points and pre-agreed actions and assist in monitoring and evaluating any impacts from the introduction. Example reference points and pre-agreed actions to be considered for the transfer of GIFT are listed in Annex 1.

Although specific adverse impacts from transferring GIFT to Nigeria are impossible to determine at present, it is logical and wise to protect native gene pools for the long-term viability of the species. Much of the justification for conserving native gene pools is to conserve genetic resources of potential value for aquaculture, so the risk to native gene pools should be reduced through risk management. The independent national advisory team, along with other experts, as necessary, should identify water bodies in Nigeria where the use of GIFT should be restricted or prevented and where conservation of native gene pools should take precedence. This may involve a survey of genetic resources in key water bodies. Anane-Taabeah et al. (2019) stated that for monitoring and evaluation to be effective countries should, "properly define their conservation goals based on the characterization of the differentiation of natural populations requiring protection from genetic introgression in specific geographic regions."

A start was made on identifying genetic resources for potential conservation in West Africa by Lind et al. (2019) and Anane-Taabeah et al. (2019) in Ghana, and this should be continued in Nigeria. WorldFish has experience in characterizing genetic resources and should incorporate this work, along with engaging key partners, into its program of work. Financing the development or protection of conservation areas in conjunction with developing areas for aquaculture, or "twinning,"[8] would help protect natural genetic diversity in tilapia species while allowing aquaculturists to farm GIFT. Twinning would help bring diverse stakeholders together for a sustainable aquaculture industry and healthy natural populations. WorldFish should also pursue twinning in its research portfolio to seek support from both the development and conservation donor groups.

Nigeria has identified four Ramsar sites that are wetlands of international significance and has pledged to conserve their important properties (Annex 2). One Ramsar site, Nguru Lake, was thought to have an endemic tilapia species—disc tilapia, though possibly *C. dageti* (Pullin, personal communication, 2021). However, details are unclear, and *C. dageti* has a wider distribution than Nguru Lake. The Ramsar sites could be areas where farming GIFT would be restricted and could be areas suitable for twinning.

4.3. Recommended procedure for introduced or transferred species that are part of current commercial practice

According to WorldFish's transfer plan (WorldFish n.d.) 10,000 yolk sac fry of GIFT will be transferred to a designated land-based, secured quarantine facility in Nigeria's Ogun State. This group of GIFT will be grown, bred and used to provide fish to other facilities in Nigeria. Currently, there is no plan for further transfers from Malaysia to Nigeria. However, several countries that have received GIFT have repeatedly asked WorldFish for additional transfers because of a lack of capacity or improper genetic resource management (Ponzoni, personal communication, 2008). If the capacity building in Ogun and Delta state breeding facilities is effective, it should not be necessary to transfer more GIFT from Malaysia in the near future.

WorldFish continues to improve GIFT at its breeding facilities in Malaysia. Therefore, in the future, it may be advantageous to commercial Nigerian aquaculture to access WorldFish's most recent and genetically improved tilapia. If such a future transfer is made, these same risk analyses should be conducted and modified with the benefit of the monitoring and research information proposed here and by other experts.

Transfer of GIFT from the breeding facilities in Ogun and Delta states to other areas in Nigeria is planned (WorldFish n.d.). As is customary in the dissemination of GIFT to countries, the receiving grow-out facilities in Nigeria should sign material transfer agreements (MTAs) indicating they will adhere to pre-agreed rules concerning further dissemination.[9] It is recognized that GIFT may become the dominant tilapia strain farmed in Nigeria and that continuous MTAs may become overly burdensome. Nonetheless, recognition of the obligations of responsible aquaculture should be made to Nigerian aquaculturists so that they can sustainably farm in the future, such as agreeing not to transfer GIFT to areas where the government has prohibited its farming.

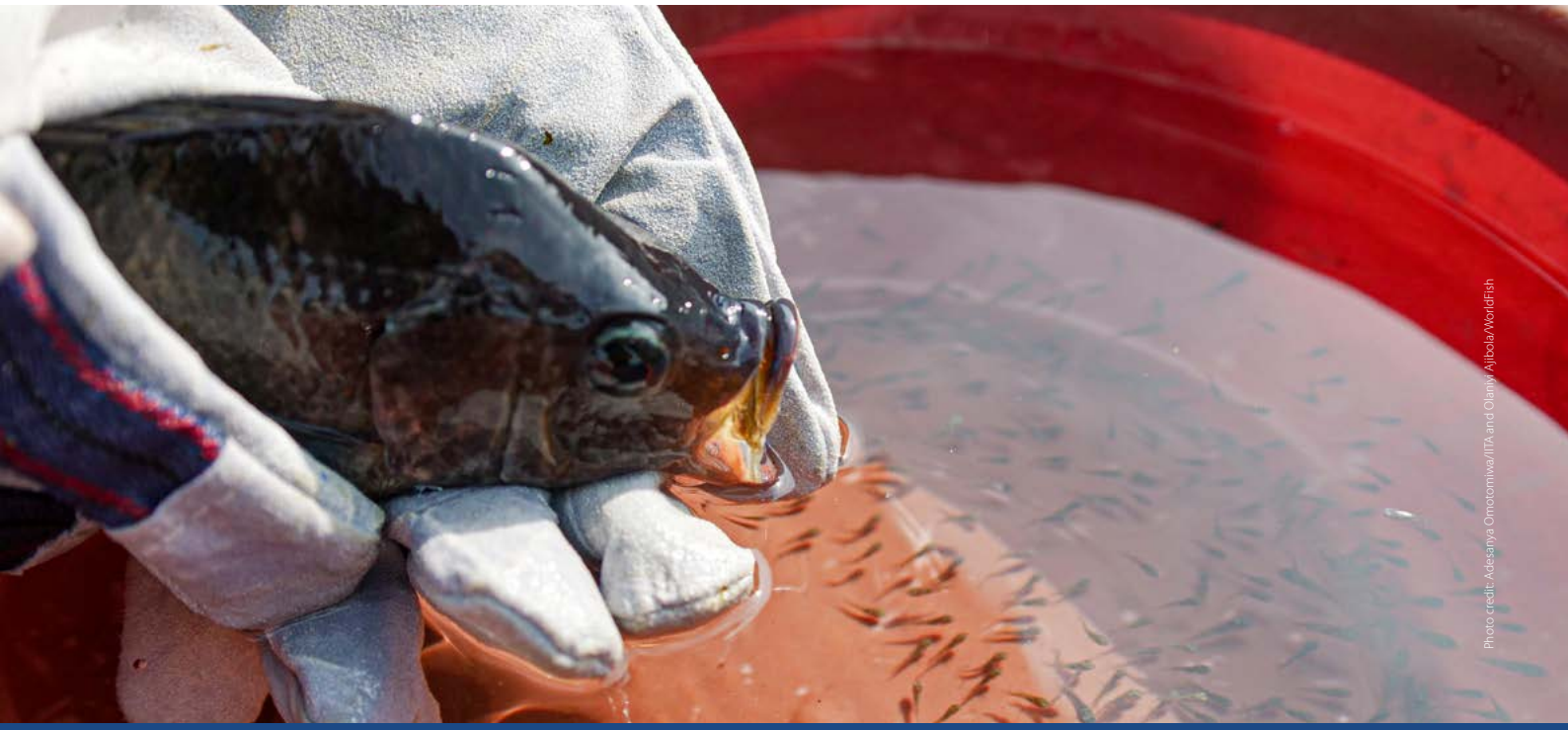


Photo credit: Adesanya Omotomiwa/IITA and Olaniyi Ajibola/WorldFish

Releasing fry from a mouth brooding tilapia (Nigeria).

5. Conclusion: Total genetic risk analysis

5.1. Risk assessment

When it is transferred to aquaculture facilities in Nigeria, it is assumed that GIFT will eventually be found in Nigerian freshwaters. There are several species in Nigeria with which GIFT could theoretically be able to breed and cause direct genetic impacts. However, there is little evidence of natural hybridization of Nile tilapia with other tilapia species in the country. In some cases, Nile tilapia has been shown to be an invasive species. Since GIFT is a strain of Nile tilapia, GIFT may also have invasive qualities in some areas. However, Nile tilapia is native to Nigeria, and GIFT is already being farmed there. Therefore, GIFT introduced through WorldFish now could have only incrementally more direct genetic impacts, if any, than those posed by native Nile tilapia and the GIFT already present. Because of this, the risk of additional serious direct genetic harm to native tilapia other than Nile tilapia is considered low.

There is a lack of specific studies on the phenotypic effects of hybridization between Nile tilapia and GIFT. As a result, it is impossible to determine at present what functional impact the change in genetic composition of native Nile tilapia would have on its long-term survival and fitness. Therefore, the genetic harm to native Nile tilapia is impossible to determine at present. However, in light of the fact that GIFT and other Nile tilapia are already being farmed in Nigeria, the incremental harm posed by the introduction of GIFT by WorldFish is considered low.

From studies on Nile tilapia in Africa, West African populations were shown to be genetically different from other African populations. Furthermore, the mating, brooding and social behavior of tilapia may promote local genetic adaptations. It is possible, then, that Nigerian populations have genetic resources that are of value to the long-term survival of the species and that may be valuable to aquaculture.

GIFT has the potential to cause indirect genetic impacts on tilapia in Nigeria through competition, predation and disease transmission. This could decrease the population size of native species, possibly leading to inbreeding depression and loss of genetic diversity and adaptive capacity. However, most tilapia species in Nigeria have wide environmental tolerances, and several have been declared potential pests. This indicates that the risk of indirect impacts reducing population size to critical levels is low in Nigeria. An exception is *S. galilaeus*, which has been listed as "near threatened" and could be further impacted by increased genetic and ecological interactions with GIFT.

5.2. Genetic risk management

Following the guidance in ICES (2005) and FAO (1996), risk of adverse genetic impacts can be reduced before GIFT enters Nigeria by convening an independent national advisory team that will review the proposal to import GIFT into the country. The team should ask WorldFish to confirm the genetic profile of the imported fish through established methods and provide a list of genetic markers or a suite of polymorphisms as described in section 2.2 to uniquely identify GIFT for subsequent monitoring activities.

It is highly probable that GIFT will breed with Nile tilapia, which will change the genetic profile of some native stocks. To reduce the risk of GIFT breeding with important native species, farms using GIFT should be located away from areas where those species are found. This would require baseline genetic information on the genetic resources of Nigerian tilapia species. Special attention should be given to the "near threatened" *S. galilaeus*.

In light of the unknown impact of changing the genetic profile of native tilapia species, a precautionary approach should be followed. It should include establishing limit and target reference points and pre-agreed actions to be followed when those reference points are reached and a monitoring system to track progress toward the reference points. This is in essence adaptive management.

Once GIFT has been introduced into Nigeria, genetic monitoring of farmed groups should be made periodically in conjunction with regular

health checks to ensure genetic variation is not being lost or compromised. A strategic monitoring system, using the markers previously supplied by WorldFish, should be established around the farms where GIFT has been transferred.

To reduce the chance of unauthorized distribution of GIFT, MTAs should be signed between the main breeding centers and the grow-out facilities.

Capacity building and awareness raising of the risks of poor genetic resource management and unauthorized movement of GIFT will help prevent loss of the genetic qualities that make GIFT such a good farmed species. They will also reduce the chance of GIFT being spread to ecologically sensitive areas or areas containing important native genetic resources.

In light of the lack of information on the genetic impacts of GIFT on local populations of Nile tilapia, the increased popularity of using GIFT in Africa and World Fish's expertise in genetic resource improvement and management, WorldFish should strive to make this introduction of GIFT to Nigeria a case study of best practices for species transfers to be included in its research portfolio. It is recognized that additional funding and partnerships will be required, and these should also be pursued as WorldFish should not be expected to conduct this innovative research alone.

It is often difficult to differentiate harm caused by genetic effects from harm caused by ecological and disease effects. As such, this genetic risk assessment should be considered in conjunction with the other risk assessments to develop an overall risk analysis of transferring GIFT to Nigeria for aquaculture purposes.

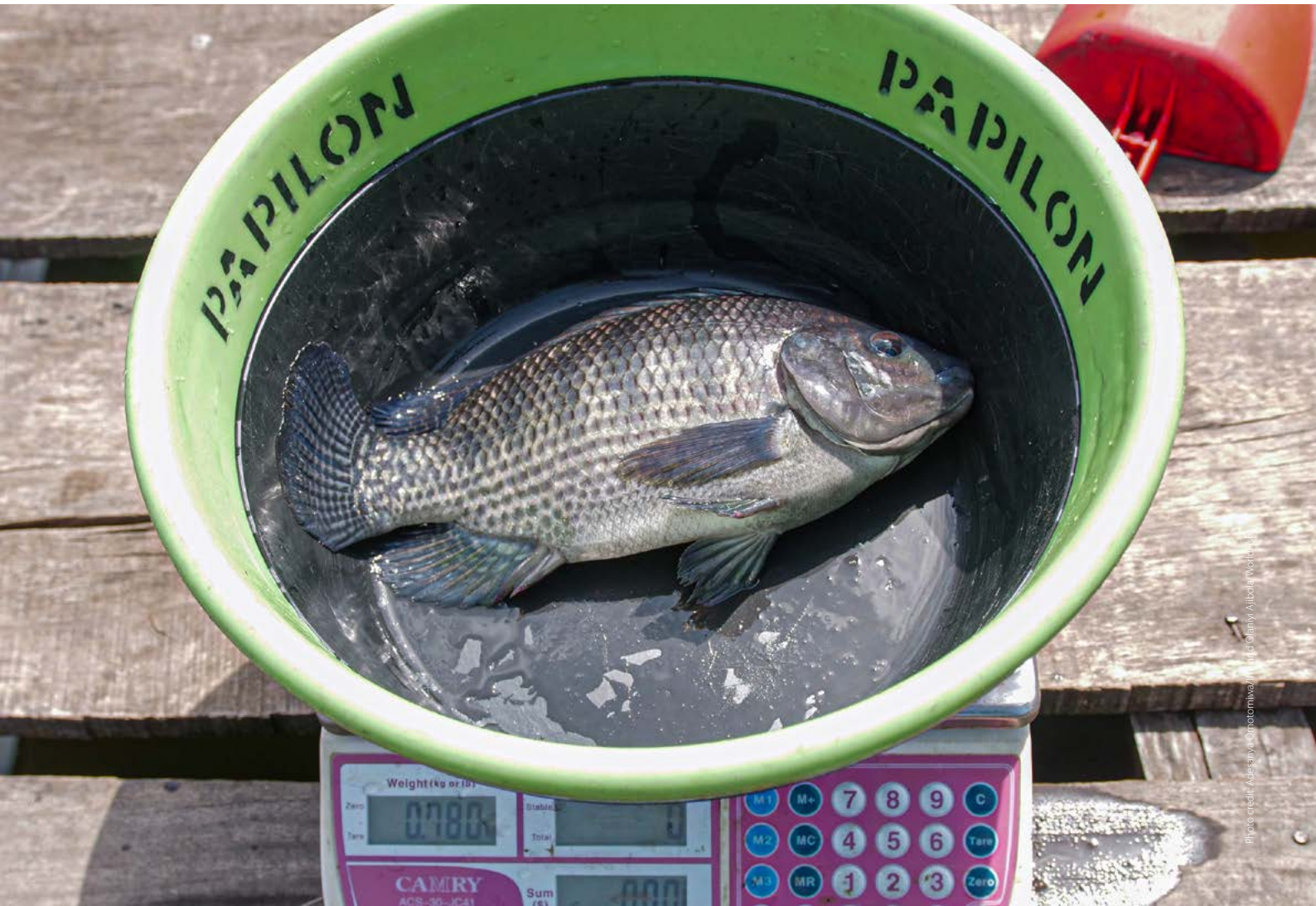


Photo credit: Adesanya Onotomiwa and Oniyi Ajibola (WorldFish)

Weighing the tilapia harvest (Nigeria).

Notes

[1] See references in Canonico et al. 2005.

[2] https://www.fishbase.de/Summary/SpeciesSummary.php?ID=2&AT=Nile+tilapia

[3] See references in Lind et al. 2019.

[4] Personal observation.

[5] See references in Eknath and Hulata 2009.

[6] See references in Eknath and Hulata 2009.

[7] Personal observation.

[8] See section 4.6 in Bartley et al. 2007.

[9] (See, for example, Annex 5.1 in FAO 2008.) WorldFish has been updating MTAs (Benzie, personal communication, 2015) and may have more current versions available.

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Annex 1. Possible reference points and pre-agreed actions for introducing GIFT into Nigeria

The precautionary approach, also known as "adaptive management," provides a useful framework for moving forward with aquaculture development in the face of uncertain risks from that development. The approach calls for establishing target and limit reference points and pre-agreed actions to be taken when the reference points are reached or breached. Target reference points indicate positive outcomes, such as maximum sustainable yield, while limit reference points indicate a boundary condition, such as a dangerous level of inbreeding. Pre-agreed actions can include either restrictions on development actions, including moratoriums, or an expansion of development activities. A monitoring and reporting system will be required to follow the progress toward the reference points.

The independent national advisory team should develop reference points and agree on contingency plans, specifically pre-agreed actions, with aquaculturists who are authorized to farm GIFT. In Table 2, indicative, but not exhaustive, actions, reference points, pre-agreed actions and the entities responsible for the actions are listed, which the independent national advisory team could modify and/or develop further. It is recognized that funds may be necessary to fulfill the actions listed, and WorldFish, along with the Government of Nigeria and other partners, should seek additional resources through project proposals and other means as necessary.

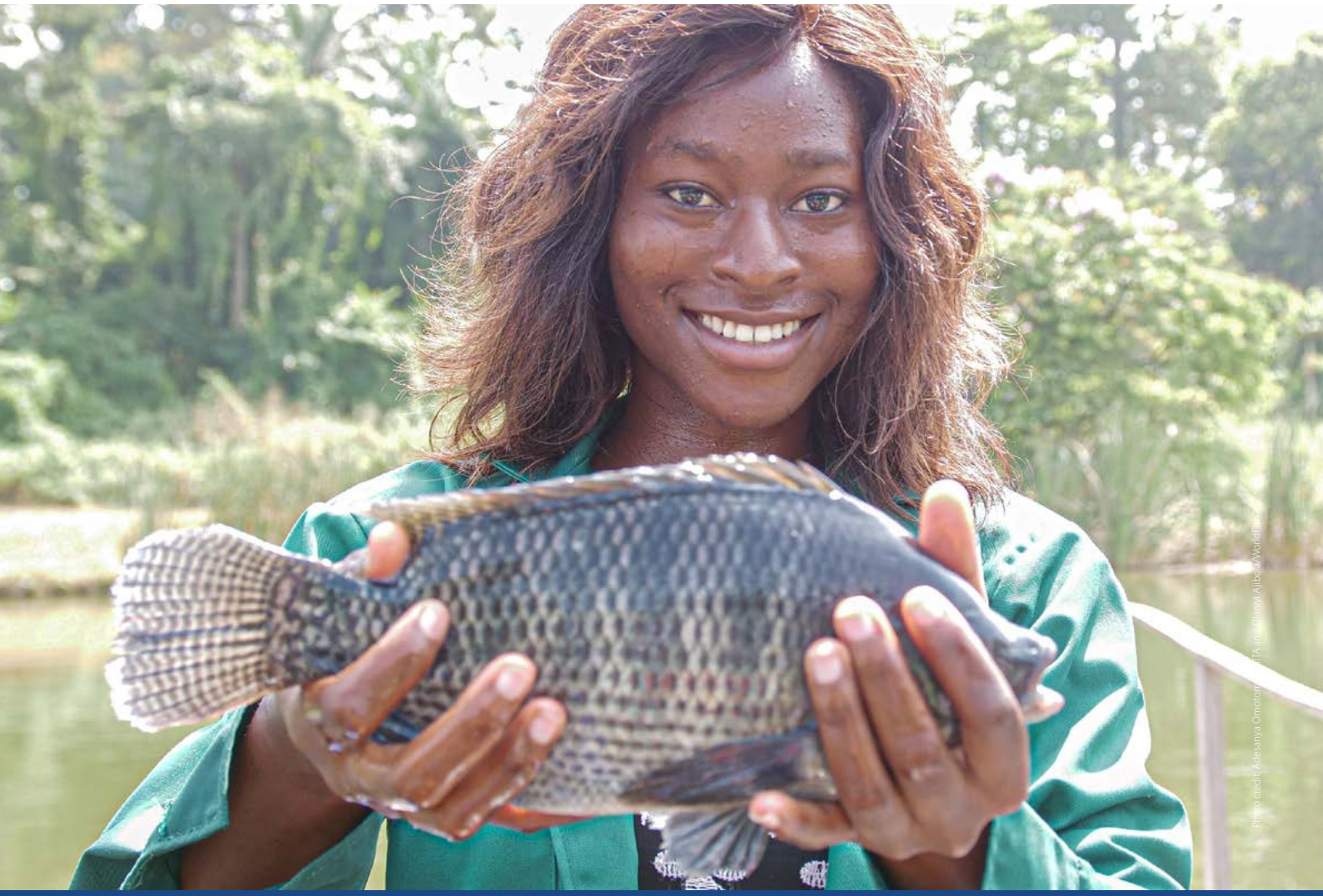

Photo credit: Adeyanya Omotoso/IITA and Olaniyi Ajiboa/WorldFish

Happy youth entrepreneur (Nigeria).

Possible actions, target (T) and limit (L) reference points, pre-agreed actions and entities responsible for implementation to reduce the risk of GIFT introductions to Nigeria				
Pre-border	**Actions**	**Reference point**	**Pre-agreed actions**	**Responsible entity**
Confirm genetic diversity of GIFT	Assess genetic markers for GIFT in broodstock and batch of fish to be imported	1. <100% GIFT strain in sample (L) 2. 100% GIFT strain in sample (T)	1. Provide alternate batch of GIFT 2. Proceed with transfer	WorldFish geneticists
Protect native genetic diversity	Survey natural populations of tilapia, and identify important tilapia genetic resources	90% of areas planned for use of GIFT surveyed (T)	Increase survey to reach 90%	Government of Nigeria resource managers
Post-border at breeding facility	**Actions**	**Reference points**	**Pre-agreed actions**	**Responsible entity**
Avoid loss of genetic diversity in broodstock and resulting progeny	Monitor genetic diversity of broodstock and progeny	1. Effective population size does not decrease by more than 5% (L) 2. Level of inbreeding remains below 12.5% (T)	Change breeding program or import more GIFT to increase effective population size and genetic diversity	Nigerian aquaculturists with agreement from the Nigerian government and WorldFish
Follow best practices in breeding	Training in broodstock management	1. 70% of hatchery staff trained (L) 2. 100% of hatchery staff trained (T)	1. Increase training 2. Discontinue training	WorldFish fish breeders and geneticists
Protect native genetic diversity	Survey local water bodies for GIFT genetic markers	1. GIFT genetic markers in the wild (L) 2. GIFT markers in new larvae in the wild (L) 3. No GIFT markers in the wild after 2 years (T)	1. Increase biosecurity 2. Suspend GIFT farming 3. Increase GIFT farming	Nigerian aquaculturists with agreement from the Nigerian government and WorldFish
Increase awareness of the value of genetic resources	Educational programs/ brochures	Distribution of material to 100% of hatchery staff (T)	Cease publication and distribution	WorldFish
Post-border at grow-out facility	**Actions**	**Reference points**	**Pre-agreed actions**	**Responsible entity**
Reduce chance of unauthorized movement of GIFT	Adopt MTAs	MTAs signed by all grow-out facilities (T)	Do not distribute GIFT until MTAs signed	Nigerian aquaculturists
Follow best practices in grow-out	Training in aquaculture management	<100% of aquaculture staff trained (L)	Increase training	Nigerian extension agents with assistance from WorldFish
Protect native genetic diversity	Survey local water bodies for GIFT genetic markers	1. GIFT genetic markers in the wild (L) 2. GIFT markers in new larvae in the wild (L) 3. No GIFT markers found in wild populations after 2 years (T)	1. Increase biosecurity 2. Suspend GIFT farming 3. Increase GIFT farming	Nigerian resource managers with assistance from WorldFish and aquaculturists
Increase awareness of the value of genetic resources	Educational programs/ brochures	Distribution of material to <100% of aquaculture staff (L)	Increase publication and distribution	WorldFish

Table 2. Genetic risk mitigation.

Annex 2. Ramsar sites in Nigeria

The following were extracted from Ramsar (2020).

Lake Chad Wetlands in Nigeria
Date of designation as Ramsar site: April 30, 2008
State: Borno
Area: 607,354 ha
Location: 13°04'N 013°48'E
Ramsar site number: 1749
Most recent RIS information: 2008
URL: https://rsis.ramsar.org/ris/1749

Description: This site is in northeastern Nigeria, bordered by Niger to the north, Chad to the northeast and Cameroon to the south. It comprises a disjointed complex of permanent freshwater marshes (formerly inundated as part of Lake Chad), some rivers and their deltas, and the remaining part of Lake Chad. The main feature, Lake Chad, is a historically large, shallow lake whose size has varied greatly over the centuries. The major vegetation types include grasses, sedges, floating macrophytes and shrubs, which form important habitats for a great variety of Palearctic migrating waterbirds, including the vulnerable marbled teal. The lake supports some indigenous fish species and is economically important, providing water, fish and other resources to the surrounding populations. Agriculture is also greatly practiced around the wetlands. Threats to the site include recession of lake waters due to climatic influence and upstream dam construction, and the consequent continuing desiccation of the wetlands. The only element of management in the area is provided by the Kanuri traditional rulers, who see to the sale of fishing rights in ponds and stretches of water as well as farming rights on the receding lakebed.

Nguru Lake (and Marma Channel) complex
Date of designation as Ramsar site: October 2, 2000
State: Jigawa
Area: 58,100 ha
Location: 10°22'N 012°46'E
Ramsar site number: 1039
Most recent RIS information: 2000
URL: https://rsis.ramsar.org/ris/1039

Description: This site is a Sahelian floodplain and lake that qualifies under the following criteria: (a) the representative criterion of embodying all diverse flora and fauna of both the Sahel and Sudan, (b) the 20,000 waterfowl criterion for at least three species (*Philomachus pugnax, Anas querquedula* and *Dendrocygna viduata*), and (c) the fish criteria, with some 20% of the fish variety of the Lake Chad Basin and about 1% of all fish caught in inland freshwater bodies in Nigeria ("disc tilapia" is thought to be endemic). Floods in the wet season play a critical role in recharging groundwater, upon which the town of Nguru and the string of settlements along the channel and lake are dependent. Some 200,000 people depend upon the site for their livelihoods, particularly for water supply. Educational research and ecotourism are practiced sustainably, but grazing, cultivation and fishing are increasingly causing pressure. The spread of invasive typha grass, taking over flood rice and cassava fields, blocking river channels and undermining fisheries, is seen as a major problem. The IUCN-Hadejia Nguru Wetlands Conservation Project (HNWCP) maintains research facilities and an information center and encourages ecotourism with boat rides. The government has accepted the HNWCP's use guidelines for the site as a working document.

Oguta Lake
Date of designation as Ramsar site: April 30, 2008
State: Imo
Area: 572 ha
Location: 05°42'N 006°47'E
Ramsar site number: 1757
Most recent RIS information: 2008
URL: https://rsis.ramsar.org/ris/1757

Description: This site is the largest natural freshwater lake in southeastern Nigeria, located in a natural depression within the floodplain of Niger River. Its water surface area varies from 180 to 300 ha depending on the season, and its average depth is 5.5 m. It receives perennial drainage from the Njaba, Utu and Awbuna rivers, and the lake drains into the Orashi River. The lake contains 258 species of phytoplankton in 107 genera and 40 fish species. Small scattered populations of the endangered Sclater's guenon (*Cercopithecus sclateri*) occur in some relict forests south of the lake. The lake is an important source of municipal and domestic water to the people of Oguta, but it is also the recipient of urban sewage. It is also of cultural and spiritual importance to many community members. Fishing and tourism are important socioeconomic activities in the area. Overfishing is stressing the lake, and sewage and sedimentation aided by deforestation are seen as threats, mitigated by the fact that the lake is annually flushed by floodwaters through an active outlet. The Oguta Lake Watershed Protection Project is involving local communities in revitalizing the lake and promoting sustainability.

Upper Orashi Forests
Date of designation as Ramsar site: April 30, 2008
State: Rivers
Area: 25,165 ha
Location: 04°53'N 006°30'E
Ramsar site number: 1759
Most recent RIS information: 2008
URL: https://rsis.ramsar.org/ris/1759

Description: This site is a forest reserve. A freshwater swamp forest in the central Niger Delta, it is inundated from September to November by floodwaters from the Orashi River, resulting in siltation and soil fertility augmentation. The reserve is the remnant of a small center of endemism, noted for hosting the critically endangered Sclater's guenon and endangered white-throated guenon, red colobus monkey and Heslop's pygmy hippopotamus. The site is a roost for the grey parrot (*Psittacus erithacas*) and also hosts a significant number of waterbird species whose distribution is confined to the Guinea-Congo Forest biome. The forest reserve has an official management plan. However, it is not being implemented, and a more articulate management plan and management structure are recommended for the reserve. Ethnic militancy and insecurity are currently hampering opportunities for tourism, education and research, while poaching and uncontrolled logging are serious related problems.

WorldFish

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.

For more information, please visit www.worldfishcenter.org