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The GIFT that keeps on giving? A genetic audit of the Fijian Genetically Improved Farmed Tilapia (GIFT) broodstock nucleus 20 years after introduction

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ABSTRACT

The Genetically Improved Farmed Tilapia (GIFT) strain of Nile tilapia is a valuable global freshwater aquaculture commodity, forming the basis of the Fiji Islands' largest freshwater aquaculture industry. Unfortunately, recent negative stock performance has been reported by farmers, possibly indicating reduced genetic diversity and inbreeding in the primary broodstock nucleus. Using high-resolution genome-wide markers (5208 SNPs), 282 individuals from three Fijian broodstock ponds were analysed and compared against two reference strains of Nile tilapia: 9th generation GIFT fish from the WorldFish Center, Malaysia, and 11th generation fish from the Abbassa Selection Line, Eygpt, (n = 94 respectively for each strain). Genetic data were used to evaluate levels of genetic diversity, inbreeding, relatedness and genetic structure; and assess the viability of the Fijian nucleus for future seed production. Results revealed only mild declines in the Fijian GIFT nucleus genetic diversity compared to both reference strains, since introduction 20 years ago. Average observed and expected heterozygosities were largely comparable for all sample groups, except for one Fijian pond which showed a heterozygote deficit (H_o = 0.2025, $H_{n.b.}$ = 0.2320). One of the three Fijian ponds sampled exhibited reduced effective population size; (N_{eLD} = 3.2 [95% C.I. = 3.2–3.2], cf. 23.3[23.2–23.3] and 31.5[31.4–31.6]), however allelic diversity remained high (A = 1.953, cf. 1.765–1.770). Fish sampled from this pond also showed a loss of rare alleles (Ar = 0.1542, cf. 0.4063–0.4065) and displayed genetic sub-structuring, possibly as a result of wild *O. niloticus* entering the broodstock nucleus. Analyses of genetic structure and relatedness revealed admixture of founding individuals, likely due to a combination of stock management practices and past pond flooding events. These findings suggest that the Fijian GIFT nucleus has retained much of the genetic diversity from its source population. It is recommended that the nucleus culture performance (fecundity, growth and survival) be evaluated through a phenotypic audit, to determine if valuable high-performing alleles have been lost. Over the longer term, stock management guidelines and genetic monitoring of the broodstock nucleus at regular intervals are proposed, to minimise further erosion of valuable genetic diversity. These results have important implications for stock management practices by demonstrating the importance of monitoring, and undertaking genetic assessments of broodstock nuclei after initial introduction, to ensure that genetic quality and performance is maintained over subsequent generations.

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1. Introduction

The Genetically Improved Farmed Tilapia (GIFT) is a selectively bred strain of the Nile tilapia (*Oreochromis niloticus*), originally developed by the WorldFish Center (WFC) in the Philippines, and subsequently Malaysia. The Nile tilapia is a globally important freshwater aquaculture commodity, particularly in developing countries in Asia, Latin America and the Pacific region (Fitzsimmons et al., 2011). Its fast growth rates, wide tolerance of environmental conditions, ease of seed production, and ready marketability among other attributes, make it highly suitable for aquaculture development and genetic improvement (Gupta and Acosta, 2004; McKinna et al., 2010).

GIFT tilapia were developed from four wild strains of *O. niloticus* sourced from the species' natural distribution, together with four high-performing cultured strains (Bentsen et al., 2016; Eknath et al., 1993). The original synthetic GIFT base population was found to be 60% heavier at harvest compared to the previous *O. niloticus* strain grown in the Philippines (Eknath et al., 1993). Following stock improvement using within- and between-family selection targeting growth rate and delayed sexual maturation, the GIFT line demonstrated a cumulative genetic gain of 67–88% (Bentsen et al., 2016; Eknath et al., 1993; Gupta and Acosta, 2004; McKinna et al., 2010). Further selective breeding was continued to propagate the line, and from the second generation onwards, GIFT fish were disseminated to several Asian and Pacific Island countries for culture, including the 7th generation line to Fiji in 1998 (Recometa-Velasco et al., 2021).

Currently, the GIFT tilapia is the most widely farmed freshwater fish in Fiji, with approximately 175 farmers involved from extensive to semi-intensive levels of culture. The development of tilapia culture has contributed substantially to inland fisheries productivity in the country, with harvests of approximately 95.5 metric tonnes/year valued at FJ $485,000.00 (Simos, 2012). Declines in stock performance had been reported (primarily early sexual maturity, depressed growth rates and reduced harvest weights and sizes), which prompted a genetic audit of Fijian GIFT stock held at the Naduruloulou Research Station (NRS) by McKinna et al. (2010). NRS is a broodstock maintenance, improvement and research facility, which also mass produces and disseminates tilapia fry to Fijian farmers. Results of this work reported reduced genetic diversity and increased levels of inbreeding in Fijian GIFT fish, compared to a GIFT reference strain, which was maintained to maximise genetic integrity (mtDNA $\theta_{\pi} = 20.9$ vs. 23.3). It was suggested that a lack of active management of the GIFT germplasm since introduction had resulted in erosion of genetic diversity, with the recommendation that a new GIFT line be considered for introduction to maintain the long-term productivity of the Fijian tilapia industry (McKinna et al., 2010).

Since then, anecdotal reports of reduced harvest weights and earlier sexual maturity (S. Mario, *pers. comm.* 2017) suggest that the performance of farmed GIFT fish in Fiji may have further deteriorated. The previous sole effort to assess the genetic quality of Fijian GIFT fish by McKinna et al. (2010) was carried out over eight years ago using markers with lower resolving power compared to genome-wide loci, and up to date information on current levels of genetic diversity for informing stock management best practices remains lacking. Maintaining high levels of genetic diversity is critical for the success of selective breeding programmes, and ensures that any deleterious effects of inbreeding and founder effects arising from closed breeding practices is minimised, with high stock fitness levels maintained (Wada and Jerry, 2008).

Given the key role played by NRS in managing the genetic quality of tilapia seed supply in Fiji, it was necessary to audit current broodstock management and fry production practices, and perform a high-resolution genetic assessment of the GIFT broodstock held there. The reports of undesirable phenotypes provided further impetus to update information on the viability of the current GIFT broodstock held at NRS. This study used a large number of genome-wide single nucleotide polymorphism (SNP) genetic markers, to provide high-resolution data on the genetic diversity, relatedness, extent of inbreeding, as well as broad and fine-scale population differentiation of Fijian GIFT broodstock *O. niloticus.* Fry and fingerling production methods at NRS were also examined, to understand the production methods employed. These data were then used to perform a viability assessment of the GIFT broodstock held at NRS for continued seed production, and to inform broodstock management practices for the Fijian tilapia aquaculture sector.

2. Methods and materials

2.1. Assessment of fingerling production practices and tissue sampling

Fisheries Officers at NRS provided information on the broodstock management and seed production systems employed for GIFT *O. niloticus*. Three ponds at NRS were selected for tissue sample collection based on NRS staff's information (see results). Two of these ponds (namely, TG-4 and RP-4) were designated as "future brooder" ponds, and contained fish descended from the originally imported seventh-generation GIFT strain stock, which are used as broodstock in the NRS hatchery facility. The third pond sampled (NP-8) was a "mass production" pond, which contained a mixture of parent and offspring fish. These ponds were deemed to represent the total broodstock pool of GIFT-only fish held at NRS.

A total of 282 individual finclip samples were collected with 94 individuals sampled per pond during August 2017. To provide a benchmark for assessment of genetic diversity and relatedness, two reference strains were also sampled, including 94 individuals each from the WorldFish GIFT ninth generation strain (Lind et al., 2017a, 2017b), and the Abbassa Selection Line (ASL), an Egyptian selectively bred strain of *O. niloticus* (ASL 11th generation, Ibrahim et al., 2013; Lind et al., 2017b). A seine net was used to aggregate tilapia in a corner of the pond where they were randomly selected for sampling. Individual sex, length and weight was recorded before finclip samples were collected. Finclips were preserved in 90% molecular-grade ethanol and submitted to Diversity Arrays Technology Ltd. (DArT PL) in Canberra, Australia, for gDNA extraction and genotyping.

2.2. DArTseq™ 1.0 library preparation, sequencing and genotype QC

Diversity Arrays Technology (DArT PL) proprietary genotyping by sequencing (DArTseq™) reduced-representation libraries were prepared as described by Kilian et al. (2012) and Sansaloni et al. (2011), with a number of modifications for the Nile tilapia genome. Briefly, genome complexity reduction was achieved with a double restriction digest, using a *Pst*I and *Sph*I methylation-sensitive restriction enzyme combination, in a joint digestion-ligation reaction at 37 °C for 2 h with 150–200 ng gDNA. Highly repetitive genomic regions were avoided and low copy regions more efficiently targeted for sequence capture with the use of methylation-sensitive REs (Elshire et al., 2011). Custom proprietary barcoded adapters (6–9 bp) were ligated to RE cut-site overhangs as per Kilian et al. (2012), with the adapters designed to modify RE cut sites following ligation, to prevent insert fragment re-digestion. The *Pst*I-compatible (forward) adapter incorporated an Illumina flowcell attachment region, sequencing primer sequence and a varying length barcode region (Kilian et al., 2012; Ren et al., 2015). The reverse adapter also contained a flowcell attachment region and was compatible with the *Sph*I cut-site overhang.

Samples were processed in batches of 94, with 15% of all samples in a batch randomly selected for replication, to provide a basis for assessing region recovery and genotyping reproducibility. Target "mixed" fragments (Ren et al., 2015), containing both *Sph*I and *Nla*III cut-sites were selectively amplified using custom-designed primers for each sample, under the following PCR conditions: initial denaturation at 94 °C for 1 min, then 30 cycles of 94 °C for 20 s, 58 °C for 30 s and 72 °C for 45 s, followed by a final extension step at 72 °C for 7 min. Amplified samples

were subsequently cleaned using a GenElute PCR Clean-up Kit (Sigma-Aldrich, cat.# NA1020-1KT), on a TECAN Freedom EVO150 automated liquid handler.

To examine fragment size concordance and digestion efficiency, all samples were visualised on a 0.8% agarose gel stained with EtBr, and quantified using the ImageJ software package (Mateos and Pérez, 2013). Samples which did not appear to have undergone complete digestion and/or amplification were removed from downstream library preparation. All samples were each normalised and pooled using an automated liquid handler (TECAN, Freedom EVO150), at equimolar ratios for sequencing on the Illumina HiSeq 2500 platform. After cluster generation and amplification (HiSeq SR Cluster Kit V4 cBOT, cat.# GD-401-4001), 77 bp single-end sequencing was performed at the DArT PL facility in Canberra, Australia.

2.3. Sequence quality control, marker filtering and genotype calling at DArT PL

Raw reads obtained following sequencing were processed using Illumina CASAVA v.1.8.2 software for initial assessment of read quality, sequence representation and generation of FASTQ files. Filtered FASTQ files were then supplied to the DArT PL proprietary software pipeline DArTtoolbox, which performed further filtering, variant calling and generated final genotypes in sequential primary and secondary workflows (Cruz et al., 2013). Within DArTtoolbox, the primary workflow first involved the package DArTsoft14 to remove reads with a quality score < 25 from further processing and apply stringent filtering to the barcode region of all sequences to increase confidence in genomic region recovery. Individual samples were then de-multiplexed by barcode, and subsequently aligned and matched to catalogued sequences in both NCBI GenBank and DArTdb custom databases to check for viral and bacterial contamination, with any matches removed from further processing.

The secondary workflow employed the DArTsoft14 and KD Compute packages along with the DArTdb database, to identify polymorphisms by aligning identical reads to create clusters across all individuals sequenced. These clusters were then catalogued in DArTdb, and matched against each other to create reduced-representation loci (RRL), based on their degree of similarity and size. SNP and reference allele loci were identified within clusters and assigned the following DArT scores: “0” = reference allele homozygote, “1” = SNP allele homozygote and “2” = heterozygote, based on their frequency of occurrence. To ensure robust variant calling, all monomorphic clusters were removed, SNP loci had to be present in both allelic states (homozygous and heterozygous), and a genetic similarity matrix was produced using the first 10,000 SNPs called to assess technical replication error (Robasky et al., 2014), and exclude clusters containing tri-allelic or aberrant SNPs and over-represented sequences.

Once SNP markers had been confidently identified, each locus was assessed in the KD Compute package for homozygote and heterozygote call rate, frequency, polymorphic information content (PIC), average SNP count, read depth and repeatability, before final genotype scores were supplied by DArT PL. Following the receipt of genotypic data from DArT PL, the dataset was further filtered to retain only a single, highly informative SNP at each genomic locus. This was achieved by filtering out duplicate SNPs (possessing identical Clone IDs), according to call rate and Minor Allele Frequency (MAF; 2% across all populations). SNPs that were monomorphic across all populations only were removed. Subsequently, loci were screened for call rate (98% threshold), average Polymorphic Information Content (PIC; 1%), MAF (2%), read depth ($\geq$10) and average repeatability (95%), to retain SNPs suitable for population genomic analyses. All loci were then tested for departure from Hardy-Weinberg Equilibrium (HWE) using Arlequin v.3.5.1.3 (Excoffier et al., 2005), using an exact test with 10,000 steps in the Markov Chain and 100,000 dememorisations. The final dataset was created, which contained selectively neutral loci after markers under selection were detected and removed.

To first create a selectively neutral dataset for population genomic analyses, a filtered dataset containing 5208 SNP loci was used as the starting point for this step. Screening for F_{st} outlier loci was carried out to identify markers potentially affected by selection, genetic drift, as well as hitch-hiking loci linked to deleterious alleles accumulated as a result of inbreeding. Both BayeScan v.2.1 (Foll, 2012; Foll and Gaggiotti, 2008) and LOSITAN selection detection workbench (Antao et al., 2008) software packages were employed to identify candidate loci under selection, at False Discovery Rates (FDRs) = 0.001, 0.005, 0.01, 0.05 and 0.1 and 0.2. Verification of loci detected at each FDR was carried out using QQ plots (data not shown). The intended approach was to select loci jointly identified by both Bayescan 2.1 and LOSITAN, at the appropriate FDR threshold determined by QQ plot distribution. As these software packages employ different analytical approaches, their joint use increased the statistical confidence of F_{st} outlier detection (Kovach et al., 2012; Pujolar et al., 2014; White et al., 2010).

Candidate loci identified with high probability using both methods were considered true outliers. All loci identified by Bayescan 2.1 were also detected by LOSITAN. Given the tendency of LOSITAN to overestimate the numbers of loci under selection however (Lal et al., 2016; Narum and Hess, 2011; Nayfa and Zenger, 2016), and disagreement on an appropriate FDR threshold to apply using both methods during testing, a conservative approach was taken where LOSITAN results were disregarded, and the Bayescan 2.1 results at an FDR = 0.01 considered.

2.4. Evaluation of genomic diversity, inbreeding and population differentiation

For assessment of genomic diversity within and between sample groups (also referred to here as populations), allelic diversity indices including the average observed (H_o) and average expected heterozygosities corrected for population sample size ($H_{n.b.}$), were computed using Genetix v.4.05.2 (Belkhir et al., 1996). Genetix was also used to calculate Wright's inbreeding coefficients (F_{is}) per sample group, mean numbers of alleles per locus (A, MAF $\geq$ 5%) and rare allelic richness (Ar, MAF $\geq$ 5%). The number of private alleles (A_p, at MAF $\geq$5%) was computed with HP-RARE v.1.0 using the rarefaction method (Kalinowski, 2004), and verified with the *R* package PopGenKit (Paquette, 2012); while the average multi-locus heterozygosity (MLH) per population was determined after Slate et al. (2004). The GenAlEx package (Peakall and Smouse, 2006), was used to determine the percentage of polymorphic loci, locally common alleles (frequency $\geq$ 5%), as well as the effective number of alleles (N_{eff}). Effective population size based on the linkage disequilibrium method (N_{eLD}) was estimated for each population, using NeEstimator v.2.01 (Do et al., 2014).

2.5. Resolution of broad and fine-scale genetic structure

Pairwise F_{st} estimates for each population were calculated using Arlequin v.3.5.1.3 with 10,000 permutations (Excoffier et al., 2005), and broad-scale population genetic structure visualised by performing a Discriminant Analysis of Principal Components (DAPC) in the *R* package *adegenet* 1.4.2 (Jombart, 2008; Jombart and Ahmed, 2011; Jombart et al., 2010). The DAPC was carried out for all selectively-neutral loci, and an α-score optimisation used in determining the number of principal components to retain. Additionally, the ‘find.clusters’ function of *adegenet* was utilised to determine the optimal number of actual clusters using the Bayesian Information Criterion (BIC) method.

To reveal any fine-scale stratification between and among all individuals, network analyses were also carried out using the Netview R package (Neuditschko et al., 2012; Steinig et al., 2016). With Netview R, population networks were generated based on a shared allele 1- identity-by-state (IBS) distance matrix created in the PLINK v.1.07 toolset (Purcell et al., 2007). Each network is constructed with the super-paramagnetic clustering (SPC) algorithm and Sorting Points Into

Neighbourhoods (SPIN) software, which computes the maximum number of nearest neighbours for a given individual (Neuditschko et al., 2012; Steinig et al., 2016; Tsafrir et al., 2005). Individual networks are then visualised and edited in the Cytoscape v.2.8.3 network construction package (Smoot et al., 2011). The IBS matrices and corresponding networks were constructed at various thresholds of the maximum number of nearest neighbour (mk-NN) values between 1 and 50, after which the optimal network for representation was selected based on cluster stability (Steinig et al., 2016).

In order to estimate proportional ancestral contributions and population stratification among sample groups, the ADMIXTURE software package was used (Alexander et al., 2009; Zhou et al., 2011). ADMIXTURE employs the likelihood model utilised in the STRUCTURE analysis package (Pritchard et al., 2000); however, instead of adopting a Bayesian approach and a Markov chain Monte Carlo (MCMC) algorithm to sample posterior likelihood distributions, it applies a Maximum Likelihood method to estimate parameters (Alexander et al., 2009). Assessment of the optimal k threshold was carried out by examining ADMIXTURE's cross-validation (cv) error statistic, by specifying the –cv flag during computations. These cv values were then plotted against the k-value for which they were calculated, with the lowest point in the graph trendline typically inferring the optimal k-threshold.

2.6. Examination of relatedness and kinship

Family relationships among all individuals were assessed with COLONY v.2.0.5.8 (Jones and Wang, 2010), to allow for the identification of any parent-offspring, full-sib or half-sib pairs present. The original filtered dataset containing 5208 SNPs was further pruned for all COLONY analyses by selecting markers that had 100% call rate across all individuals, generating a dataset containing 1876 SNPs. This dataset was then divided into three subgroups containing all NRS broodstock individuals only, the WorldFish GIFT ninth-generation reference strain and the ASL reference strain respectively. All NRS individuals were grouped together as the results of earlier analyses examining genetic structure (see results), had discovered substantial relatedness among and between ponds, necessitating that they be examined together. Each NRS sample group was also analysed separately for comparison.

COLONY computations were run without updating allele frequencies with run progression, specifying the presence of inbreeding, specifying polygamy for both males and females, not inferring clones and scaling full sib-ship relationships. For prior settings, weak sib-ship priors for all relationship determinations were requested, population allele frequency was specified as unknown (i.e. calculated during the run), the full-likelihood (FL) method was used for all runs and the option for medium length runs at high precision selected. A total number of three runs were carried out, each using a different random number seed, all assuming a genotyping error rate of 0.01. All other options remained at their default settings.

The numbers of full-sib and half-sib dyads detected during each run were first ordered by probability, and then tallied for each sample group using an inclusion threshold of $p \leq 0.01$. The best (ML or Maximum Likelihood) full-sib families were identified by the probability ($p \leq 0.01$) for the inclusion of individuals in that family. The total number of best full-sib families was reported for each sample group, which was also broken down into the number of families where two or more members had been identified, and singletons, where only one member was identified within the respective sample group. The numbers of best (Maximum Likelihood) family clusters were also reported for all sample groups by ordering according to a probability threshold of $p \geq 0.01$, and tallying the number of different cluster indices determined by COLONY. Putative parents were identified only within these highly probably clusters ($p \leq 0.01$), by determining the numbers of unique Mother and Father IDs (Jones and Wang, 2010). Within the NRS broodstock sample group, to examine the distribution of families across each of the ponds sampled, all of the best (ML) full-sib families identified by COLONY were separated by individual according to pond number, and the result graphed.

Pairwise relatedness within and between population groups was assessed using the COANCESTRY v.1.0.1.9 package (Wang, 2011). The re-filtered dataset of 1876 SNPs was again employed to estimate relatedness (r) coefficients, using the triadic likelihood estimator, which accounts for inbreeding (Taylor, 2015; Wang, 2007) (TrioML option selected). Bootstrapping ($n = 1000$) was also implemented to generate 95% confidence interval limits for the final reported estimates.

3. Results

3.1. Assessment of fingerling production practices and broodstock tissue sampling for genetic analyses

The fry and fingerling production systems employed for GIFT *O. niloticus* at NRS are complex, with three seemingly independent systems operating in parallel, drawing upon sexually mature fish sourced from the "future broodstock pool" (see Appendix A). Anecdotal reports by NRS staff indicated that a major flooding event over November–December 2017 at NRS due to inclement weather, resulted in fish from all broodstock production systems becoming mixed. This was an important consideration for deciding which broodstock systems and ponds to sample for the genetic audit. To carry out representative tissue sampling of all NRS broodstock, samples were obtained from the future broodstock pool and the mass production pond system, as these directly produce fry for distribution to farmers.

It is evident that the future broodstock pool of fish (see Appendix A) is central for the provision of broodstock for use in the three fry production systems employed at NRS, however it wasn't apparent if this pool of fish itself receives any management action to maintain genetic diversity and minimise inbreeding. Reports by NRS staff indicate that broodstock routinely used in the knockdown tank hatchery system are culled once their reproductive output declines, and broodstock are replenished from the future brooder pool. Periodically, fry produced in the knockdown tank hatchery system are retained to add to the future brooder pool, and perhaps this is the only mechanism by which fish are added to the broodstock group. This practice is a concern, as over time the effective population size of the entire broodstock pool will be reduced, and inbreeding rates increase with the mating of closely related individuals. Also of concern is the genetic diversity of fish maintained in the mass production system, which reportedly sees no management action to cull older individuals and replenish reproductively active fish. These individuals are likely over successive generations to experience deleterious inbreeding effects, and consequently produce poorer quality fingerlings that may impact farm productivity.

3.2. Morphometric data

Of the 282 GIFT fish sampled, 218 were males and the remaining 64 female. Overall, higher proportions of male compared to female fish were sampled from each pond (Table 1). Length-weight relationships for both sexes are presented in Fig. 1 for each respective pond, while the numbers of all fish sampled and their weight distributions are provided in Fig. 2. The total numbers of individuals stocked were 2500 for pond TG-4, 740 for NP-8 and 1500 for RP-4. A large number of fingerlings of various sizes and fry were also observed in each pond, indicating the presence of several generations. Averaged standard lengths over all three ponds differed slightly (0.3 cm) between males and females, with females being slightly longer at 12.2 cm. A similar difference of 0.1 cm in averaged total lengths between males and females was found, with females being slightly longer at 14.9 cm. The largest individuals sampled (in terms of standard and total lengths) were males, at 26.5 cm and 34.0 cm respectively.

Length-weight distributions differed between ponds (Fig. 1), with comparatively even length patterns observed between males and

Table 1
Weight and standard length data summary of NRS GIFT broodstock *O. niloticus* sampled.

	Pond TG-4		Pond RP-4		Pond NP-8	
	Female	Male	Female	Male	Female	Male
n	37	57	19	75	8	86
Average length ± SD (cm)	13.2 ± 1.4	13.8 ± 2.0	10.5 ± 2.2	10.2 ± 2.1	11.5 ± 0.6	12.2 ± 2.5
Median length (cm)	12.9	13.4	9.8	9.7	11.5	11.9
Average weight ± SD (g)	86.5 ± 28.3	103.7 ± 57.4	51.0 ± 34.5	46.7 ± 31.5	53.7 ± 7.2	76.1 ± 79.7
Median weight (g)	80.4	89.8	35.8	34.0	54.6	57.8

females within all ponds (Fig. 1a, b and Table 1). However, males were slightly longer than females in pond NP-8, (12.2 cm c.f. 11.5 cm average lengths respectively, Fig. 1c). Of all ponds, RP-4 contained the smallest fish, with median lengths of 9.8 cm and 9.7 cm for females and males respectively (Table 1), while TG-4 contained the largest (12.9 cm and 13.4 cm female and male respective median lengths). Weight trends between ponds showed that males were heavier, with the exception of pond RP-4, where comparable weights were recorded (35.8 g and 34.0 g female and male median weights respectively). Pond RP-4 also contained the lightest fish (average weights of 51.0 g and 46.7 g for females and males respectively), while the heaviest fish were sampled from TG-4 (average weights of 86.5 g and 103.7 g for females and males, respectively).

Examination of weight distributions and the numbers of individuals sampled from discrete fish weight classes revealed that a much higher proportion of males were collected than females, across all weight classes (Fig. 2). The majority of male fish sampled fell into a lower spread of weight class bins (ranging from <20 g to ~100 g), whereas females occupied higher weight class bins (range of ~30 g–140 g).

3.3. DArTseq™ 1.0 genotyping and marker filtering

A total of 282 NRS broodstock tissue samples were supplied to DArT PL for genotyping, however it became necessary to exclude two individuals due to gDNA quality issues. The remaining 280 individuals were co-analysed post-sequencing with reference samples including 94 WorldFish Centre ninth-generation GIFT fish, as well as 94 fish from the Egyptian ASL strain. Consequently, the raw dataset contained a total of 33,156 SNPs genotyped across all 468 individuals, at call rates ranging from 38 to 100%. The first filtering step was undertaken to remove duplicate (clone) SNPs at genomic loci, and resulted in the removal of 4113 SNPs (12% loss), after which the dataset was filtered for call rate (98%), average PIC (1%), MAF (2%) and average repeatability (95%). All loci called at a read depth < 10 were also excluded from the filtered dataset. A total of 2 loci were found to deviate from HWE ($p < 0.009$), and 163 loci were monomorphic across all sample groups, which were subsequently removed. These steps collectively resulted in the retention of 5229 SNPs. Testing of this filtered dataset for F_{st} outlier loci detected 21 SNPs putatively under balancing or directional selection, and their removal generated a final neutral dataset of 5208 SNPs. This dataset was used for performing all population genomic analyses.

3.4. Genomic diversity and inbreeding

Population assignments for diversity analyses were made on an individual pond basis for all NRS samples, and separately for each reference strain of *O. niloticus* (WorldFish GIFT ninth-generation strain and ASL). NRS Ponds TG-4 and RP-4 were dedicated broodstock ponds, while NP-8 was a mass production pond containing both unmanaged broodstock fish and their offspring. Effective population sizes (N_{eLD}) for all NRS broodstock ponds ranged from 3.2–31.5 (Table 2), and were lower overall than values obtained for both reference sample groups (48.0 and 39.8 for the GIFT strain and ASL, respectively). The N_{eLD} of 3.2 (95% C.I. = 3.2–3.2) obtained for NRS pond RP-4 was particularly low, reflecting that founders contributing to these individuals may have experienced one or more population bottlenecks. A combined analysis of all NRS ponds excluding RP-4 produced a N_{eLD} of 29.2 (95% C.I. = 29.1–29.2).

Patterns observed in the mean numbers of alleles per locus (A, Table 2) differed from the trend in N_{eLD} however, with the highest value observed for NRS pond RP-4 ($A = 1.953$), whereas the remaining NRS ponds sampled, together with the reference GIFT strain all returned nearly identical lower values (range of $A = 1.76$–1.77). Fish sampled from NRS pond RP-4 contained all private alleles (those alleles unique to a single population), identified among the three NRS sample groups ($Ap = 0.062$, 325 private alleles detected); which was substantially higher than the reference GIFT fish ($Ap = 0.001$, 6 private alleles detected). A small proportion of private alleles (2.8%) was also detected in the ASL samples ($Ap = 0.028$, 148 private alleles detected), as was the lowest number of alleles per locus overall ($A = 1.644$). However, this is to be expected, as the Egyptian *O. niloticus* founders contributing to this strain were likely to be genetically divergent from any fish of GIFT-line origin.

Examination of the rare allelic richness (Ar) and the effective number of alleles (N_{eff}) per population largely followed the trend observed for A, with NRS ponds TG-4 and NP-8 returning very similar values to the reference GIFT fish ($Ar = 0.4065$ and 0.4063 respectively, cf. 0.4163; with $N_{eff} = 1.309$ and 1.304 respectively, cf. 1.297). For both these metrics, NRS pond RP-4 produced the lowest and highest values respectively ($Ar = 0.1542$, $N_{eff} = 1.353$), suggesting that the lower rare allelic richness and higher allelic diversity observed in these fish may originate from decreased diversity due to a genetic bottleneck, and possible genetic sub-structuring (Wahlund effect). Assessments of locally common alleles (with a frequency ≥ 5%), and proportions of polymorphic loci present in each sample group further indicate the presence of private alleles in NRS pond RP-4 broodstock, as these fish produced the highest frequency values (0.097; 95.3% polymorphic loci), compared with all other sample groups which ranged from 0.063; 76.5% polymorphic loci (NRS pond NP-8) to 0.070; 77.6% polymorphic loci (GIFT reference strain).

Average observed and expected heterozygosities were largely comparable for all sample groups, with the exception of NRS pond RP-4, indicating a heterozygote deficit ($H_o = 0.2025$, $H_{n.b.} = 0.2320$). Observed heterozygosity values obtained for both the other NRS pond samples (0.1924 and 0.1896 for TG-4 and NP-8 respectively), were similar to the value obtained for the GIFT reference strain individuals ($H_o = 0.1842$). Inbreeding coefficient values suggest mating of closely related individuals for NRS pond RP-4 ($F_{is} = 0.1277$, $p < 0.01$), consistent with the heterozygote deficit evident in H_o values for this sample group. Values of F_{is} for all remaining sample groups were positive ($p < 0.01$), ranging from 0.0037 (NRS pond TG-4) to 0.0129 (GIFT reference strain, see Table 2). For NRS pond sample groups TG-4 and NP-8, F_{is} values were lower compared to the GIFT reference strain, suggesting unmanaged breeding in these fish through random mating. Values computed for average MLH support this observation, as all NRS sample groups exhibited slightly reduced diversity (MLH range of 0.2069 to 0.2245), when compared to the GIFT reference strain (MLH = 0.2434). Individuals sampled from NRS pond RP-4 demonstrated the lowest MLH (0.2069), which was also low in comparison to the ASL reference samples (MLH = 0.2122).

3.5. Genetic structure

Pairwise F_{st} estimates were significant for all sample group comparisons, with divergence between NRS sample groups ranging from

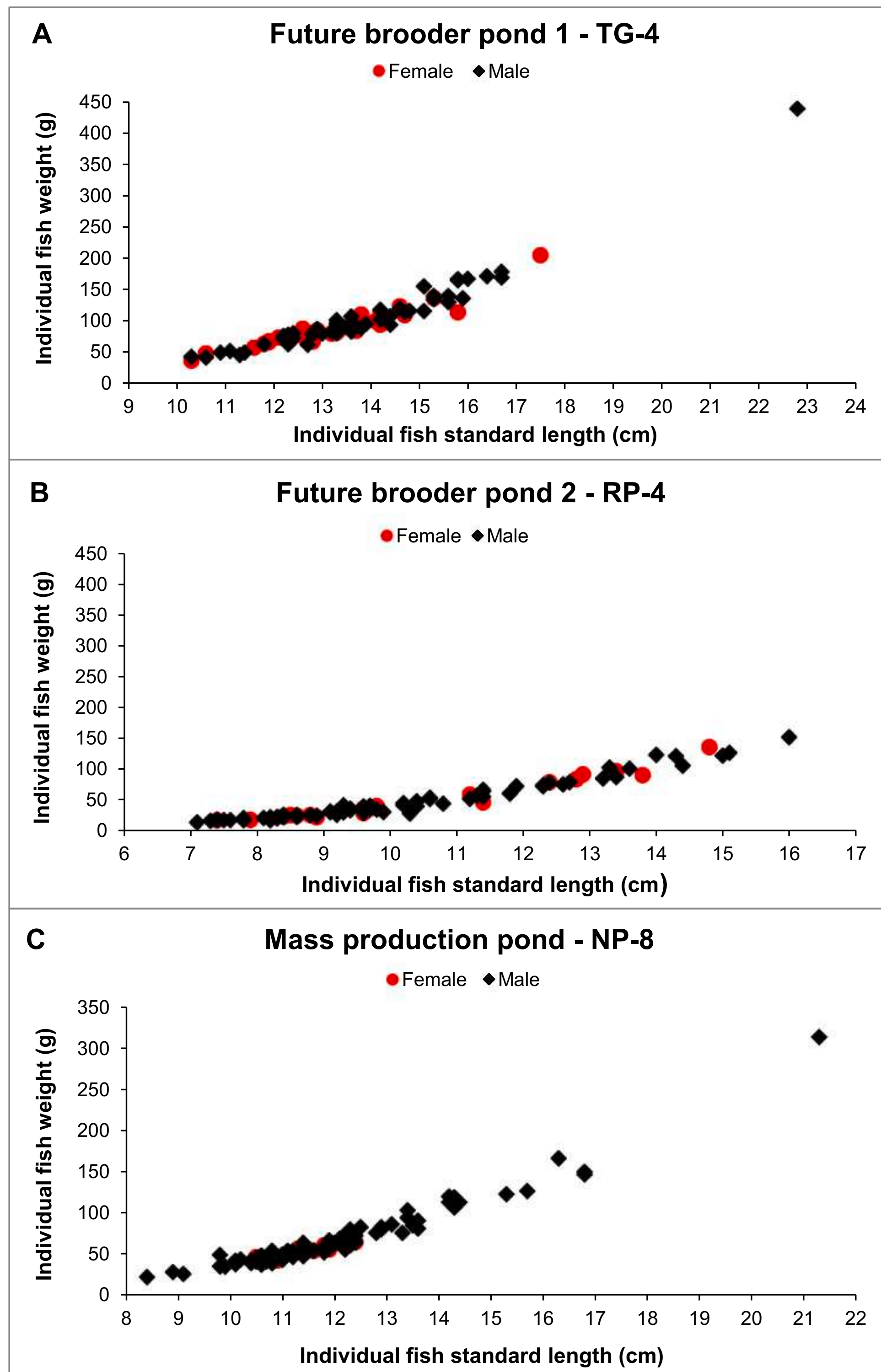

Fig. 1. Length-weight relationships of male (black diamonds) and female (red circles) broodstock GIFT *O. niloticus* sampled from three separate ponds at NRS. Panels A and B display data from fish collected from future broodstock ponds TG-4 and RP-4 respectively, and panel C data from fish sampled out of mass production pond NP-8. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

0.0036 to 0.0159 (Table 3). NRS pond RP-4 displayed the highest level of divergence from the other NRS sample groups, with pairwise comparisons between TG-4 and RP-4, and NP-8 and RP-4 producing similar levels of differentiation (pairwise F_{st} = 0.0151 and 0.0159, respectively). Conversely, this degree of separation was not evident between TG-4 and NP-8 fish (pairwise F_{st} = 0.0036). Differentiation between the NRS sample groups and the GIFT reference strain ranged between 0.0446 and 0.0493, while levels of divergence from the ASL reference strain were higher still (pairwise F_{st} range = 0.1429–0.1795). Pairwise Nei's standard genetic distances (D_S) described a similar pattern to the pairwise F_{st} estimates (Table 3), with NRS pond RP-4 fish demonstrating separation from TG-4 and NP-8 samples (D_S = 0.004 cf. 0.001), and all

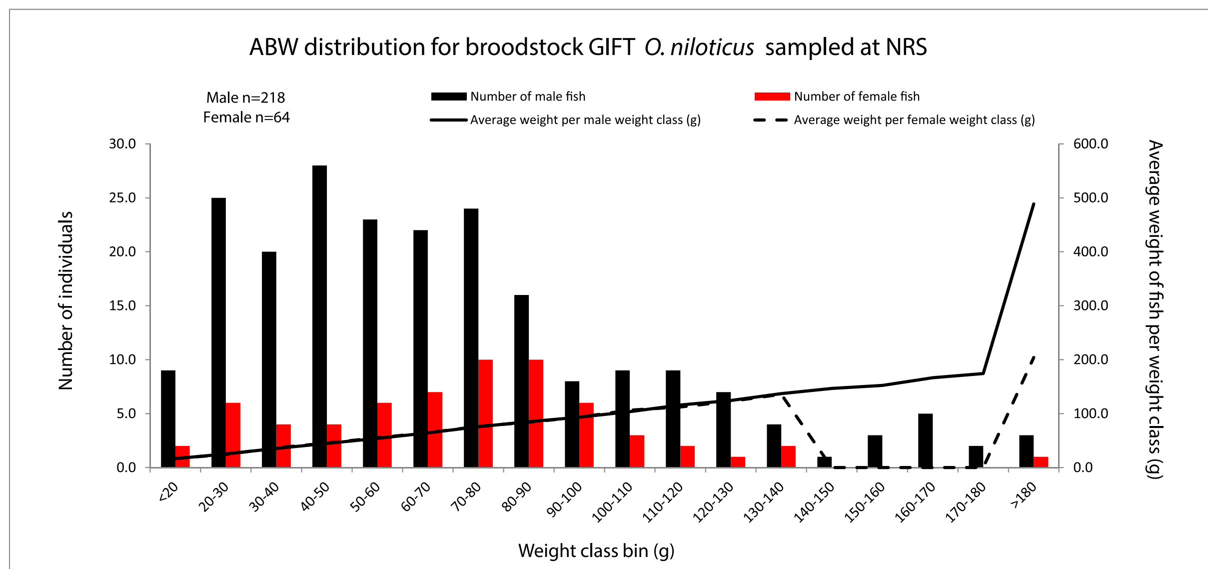

Fig. 2. Average body weight distributions of broodstock *O. niloticus* var. GIFT sampled at NRS. The numbers of fish sampled within each weight class are represented by bars for males (black) and females (red) on the left primary y-axis on the left. The average weights within each weight class are presented on the secondary y-axis on the right, represented by a solid black line for males and a broken red line for females. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Genetic diversity indices computed for the *O. niloticus* GIFT populations sampled. Parameters calculated include the effective population size by the linkage disequilibrium method (N_{eLD}; 95% confidence intervals indicated within brackets), mean number of alleles per locus (*A*), standardised private allelic richness (*Ap*, MAF ≥5%) - the total number of private alleles detected per population is shown in brackets below the *Ap* value, rare allelic richness (*Ar*, MAF ≥5%), effective number of alleles (N_{eff}), percentage of polymorphic loci, number of alleles locally common to a population found in 50% or fewer populations (MAF ≥5%), observed heterozygosity (H_o), average expected heterozygosity corrected for population sample size ($H_{n.b.}$), inbreeding coefficients (F_{is}) and average individual multi-locus heterozygosity (MLH). All computations were generated using a dataset containing 5208 genome-wide SNPs.

Population	n	N_{eLD} [95% C.I.]	*A* (≥5%)	*Ap* (≥5%)	*Ar* (≥5%)	N_{eff}	% of polymorphic loci	Locally common alleles (≥5%)	H_o (±SD)	$H_{n.b.}$ (±SD)	F_{is} (p < 0.01)	Av. MLH (±SD)
NRS future brooder pond TG-4	93	31.5 [31.4–31.6]	1.770 ± 0.006	0	0.4065	1.309 ± 0.005	76.96	0.066 ± 0.003	0.1924 ± 0.1827	0.1931 ± 0.1777	0.0037	0.2140 ± 0.0246
NRS future brooder pond RP-4	94	3.2 [3.2–3.2]	1.953 ± 0.003	0.062 ± 0.003 (325)	0.1542	1.353 ± 0.004	95.26	0.097 ± 0.004	0.2025 ± 0.1469	0.2320 ± 0.1454	0.1277	0.2069 ± 0.0252
NRS mass production pond NP-8	93	23.3 [23.2–23.3]	1.765 ± 0.006	0	0.4063	1.304 ± 0.005	76.52	0.063 ± 0.003	0.1896 ± 0.1811	0.1907 ± 0.1763	0.0060	0.2245 ± 0.0424
WorldFish GIFT reference	94	48.0 [47.9–48.2]	1.777 ± 0.006	0.001 ± 0.001 (6)	0.4163	1.297 ± 0.005	77.75	0.070 ± 0.004	0.1842 ± 0.1793	0.1866 ± 0.1758	0.0129	0.2434 ± 0.0734
Abbassa Selection Line reference	94	39.8 [39.7–40.0]	1.644 ± 0.007	0.028 ± 0.002 (148)	0.5242	1.228 ± 0.004	64.38	0.066 ± 0.003	0.1453 ± 0.1742	0.1460 ± 0.1684	0.0050	0.2122 ± 0.0379

Table 3
Pairwise population differentiation estimates computed for the GIFT and reference strains of *O. niloticus* sampled using 5208 SNPs. Pairwise F_{st} values (Weir and Cockerham's 1984 unbiased method) are reported below the diagonal, and were generated in Arlequin v3.5.1.3 following 1000 permutations and a significance threshold of p ≤ 0.001. Nei's (1978) standard genetic distances (D_S) are reported above the diagonal and were computed in Genetix v4.05.2 with 10,000 permutations.

	NRS future brooder pond TG-4	NRS future brooder pond RP-4	NRS mass production pond NP-8	WorldFish GIFT reference 9th generation	ASL reference 11th generation
NRS future brooder pond TG-4	–	0.004	0.001	0.012	0.045
NRS future brooder pond RP-4	0.0151	–	0.004	0.012	0.038
NRS mass production pond NP-8	0.0036	0.0159	–	0.012	0.045
WorldFish GIFT reference 9th generation	0.0477	0.0446	0.0493	–	0.044
ASL reference 11th generation	0.1793	0.1429	0.1786	0.1795	–

NRS sample groups differentiating from the reference GIFT and ASL samples (D_S = 0.012, and 0.038–0.045 respectively).

Visualisation of broad-scale population structure with a DAPC (following α-score optimisation which resulted in the retention of 23 principal components), revealed clear differentiation between both reference strains, and all three NRS broodstock sample groups (Fig. 3). As per patterns observed in allelic diversity, the ASL sample group was the most divergent from the NRS fish due to geographic isolation, however the ninth-generation GIFT reference individuals also displayed marked separation. Among NRS broodstock samples, individuals sampled from all three ponds remained indistinguishable from each other.

Examination of fine-scale population structure among all sample groups using Netview P networks (Fig. 4) resolved similar broad patterns of differentiation to the DAPC, but offered greater resolution at the individual level, particularly among the NRS broodstock sampled. Three large clusters were resolved, with all three NRS sample groups comprising a single, large diffuse cluster, confirming that NRS GIFT broodstock have become admixed since introduction of their founders in 1997. The remaining clusters contained the two reference strain sample groups.

The WorldFish GIFT reference strain fish clustered in greater proximity to the NRS individuals, and some GIFT reference strain individuals nested within the NRS sample cluster, reflecting genetic similarity which was not apparent in the DAPC analysis. Within the large cluster resolved for all NRS broodstock samples, several tightly linked sub-clusters were observed suggesting the presence of family groups, particularly for individuals sampled from ponds RP-4 (which had displayed signatures of reduced diversity and inbreeding in earlier analyses), and NP-8, where uncontrolled reproduction is known to have occurred.

To evaluate individual-level relationships on a per pond basis, additional networks were generated for each sample group separately (Fig. 5). Among NRS broodstock samples, fish collected from pond RP-4 resolved the smallest number of clusters of closely related individuals (Fig. 5B, n = 4), while the remaining NRS ponds TG-4 and NP-8 generated 8 and 11 clusters respectively (Fig. 5A and C). These patterns corresponded with the relative diversity levels observed for these sample groups, suggesting a higher level of relatedness for pond RP-4, compared to TG-4 and NP-8. Contrastingly, both reference strain samples resolved singular large clusters, with 3 and 2 much smaller satellite clusters for the GIFT and ASL sample groups respectively (Fig. 5D and E), reflecting controlled reproduction in the maintenance of discrete family lines.

Estimation of proportional ancestral contributions with the ADMIXTURE package (Fig. 6), indicated stratification of all sample groups into three broad groups at all k thresholds equal to and higher than k = 4. These groups comprised all NRS ponds together in one cluster, along with each of the reference strains in their own respective clusters. The ADMIXTURE cross-validation error statistic plot (see Appendix B) was not informative for this dataset in selection of the optimal k threshold (the suggestion for the optimal threshold was k = 8), however visual inspection of stratification patterns suggest that k = 4 is the best fit. Between k = 3 and k = 5, within-population stratification of individuals sampled from NRS future brooder pond RP-4 is evident, suggestive of population sub-structuring.

3.6. Relatedness and kinship

A summary of individual relatedness and sib-ship reconstruction metrics determined by COLONY is reported in Table 4 below, while pairwise population relatedness (r) estimates generated from COANCESTRY simulations are presented in Table 5. All triplicate COLONY

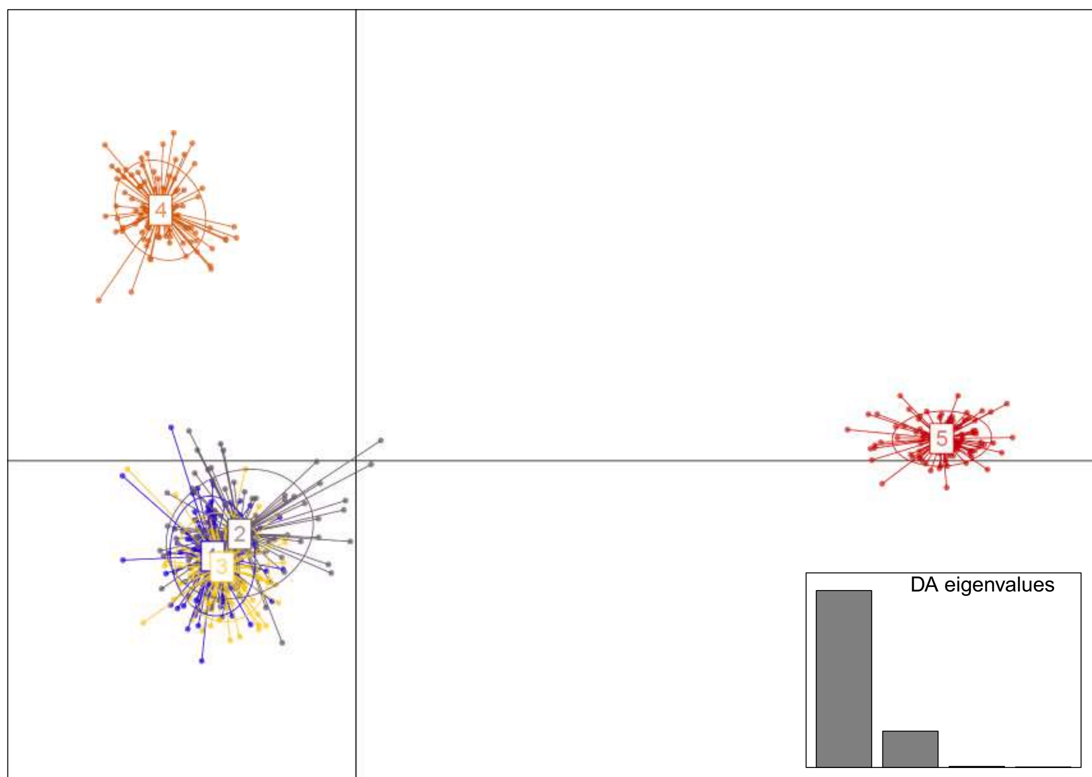

Fig. 3. Discriminant Analyses of Principal Components (DAPC) carried out using the *R* package *adegenet* to illustrate broad-scale patterns of population structure among the 468 individual of *O. niloticus* sampled. The axes represent the first two discriminant functions, respectively. Dots on scatterplots represent individuals, with colours denoting sampling origin and inclusion of 95% inertia ellipses. Sample groups 1 (blue), 2 (purple) and 3 (yellow) represent all NRS broodstock fish (ponds TG-4, RP-4 and NP-8, respectively); while 4 (orange) and 5 (red) represent the reference strains of ninth-generation WorldFish Center GIFT fish and the Egyptian Abbassa Selection Line (ASL), respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

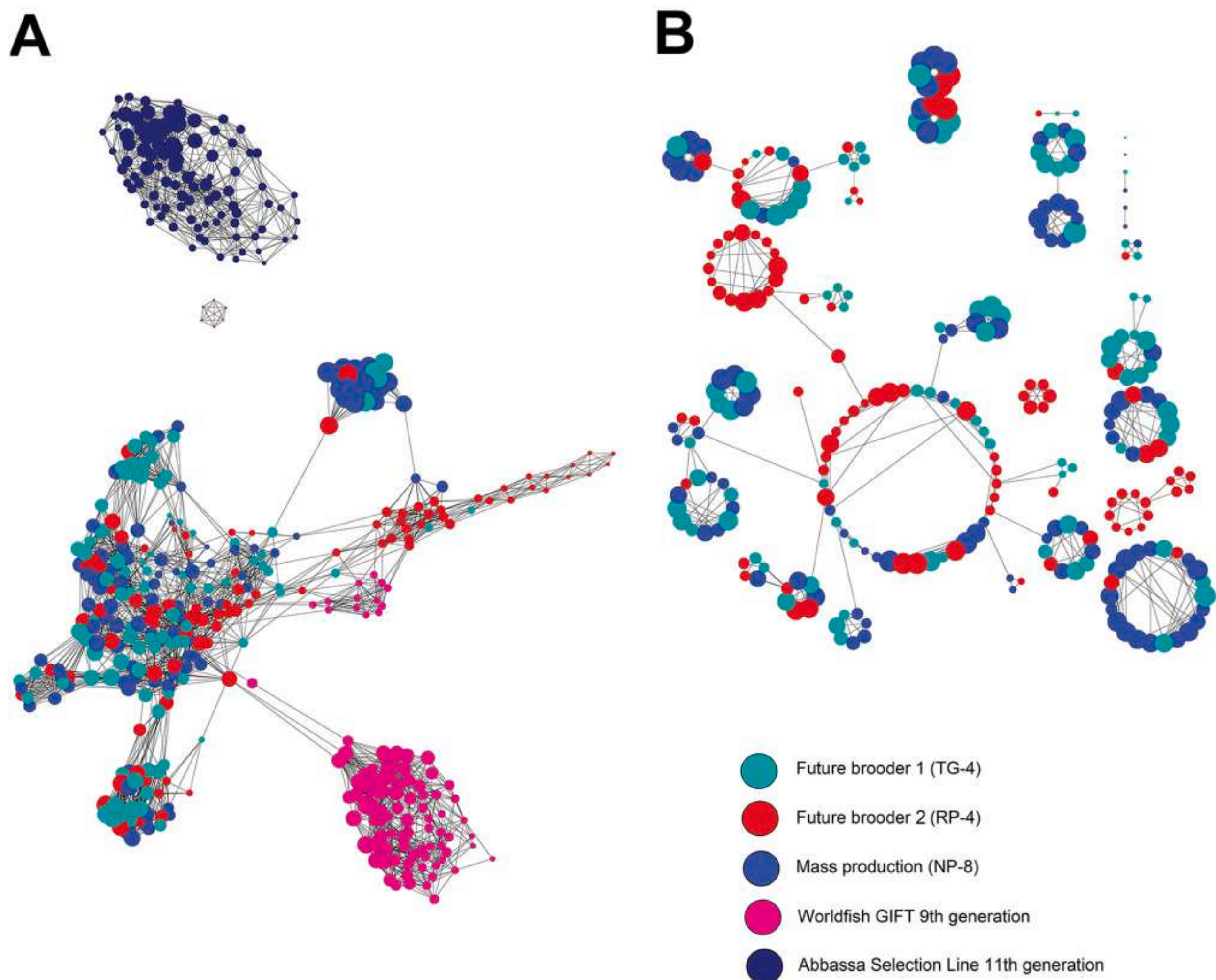

Fig. 4. Fine-scale population genetic structure networks of NRS *O. niloticus* var. GIFT broodstock groups and the two reference strains (9th generation GIFT and 11th generation ASL), analysed using Netview R. Network A was produced from all sample groups analysed collectively, and generated using an organic topology framework at mk-NN=20. Network B was produced following analysis of all NRS populations only, and generated using a circular topology framework at mk-NN=6. Both networks display node sizes mapped to the relatedness of individual fish.

runs for each respective sample group returned identical results for the metrics reported. The combined number of full-sib and half-sib dyads detected for individual NRS sample group ponds were higher (range of 139–213), compared to both reference strains (91 and 92 for the GIFT and ASL respectively). Among the NRS ponds, RP-4 contained the lowest number of full-sib and half-sib dyads detected (n = 139). When all NRS sample groups were analysed together however, the number of full-sib and half-sib dyads showed a nearly three-fold increase, reflecting the increase in the number of individuals sampled.

The number of best full-sib families detected for all individual NRS ponds was variable, ranging from 42 to 78 families, while the reference strains returned intermediate values (GIFT = 61, ASL = 53). Interestingly, the largest number of full-sib families were identified in NRS pond RP-4, with the majority of these originating from singleton family members, compared to families where two or more members were detected. Contrastingly, the family compositions of all other sample group full-sib families identified had more even contributions from families comprising singleton members, and families where two or more members were identified.

Similar trends were evident in the number of best (ML) clusters resolved, as with the combined number of full-sib and half-sib dyads. Here, the lowest number of clusters were identified in pond RP-4 (18), while larger and more comparable numbers were detected for the other NRS ponds (TG-4 = 55, NP-8 = 47). In comparison, lower numbers of clusters were identified for the reference strains (GIFT = 49, ASL = 31). These patterns suggest that while a smaller overall number of families is likely to be present in pond RP-4 compared to the other NRS sample groups, these families are more divergent from each other, than those families present in the other NRS sample groups.

As parentage information and parental genotypes were not available for the current study, male and female polygamy had to be assumed for all COLONY runs. Despite this limitation, examination of the number of best clusters allowed determination of putative parentage, which was found to be relatively even for mothers and fathers for all sample groups (Table 4), but skewed for NRS pond TG-4 (higher number of fathers). This result could reflect uncontrolled reproduction within that pond. Examination of the distribution of members belonging to the 40 best full-sib families identified among all NRS broodstock (Fig. 7), reflects a high degree of admixture of the broodstock pool, supporting earlier determinations of genetic structure (Figs.3, 4, 6). Over half (52.5%) of these 40 families were sampled from two ponds, while 25% were collected from all three ponds sampled.

Pairwise relatedness estimates between populations (Table 5) displayed concordant trends identified by genetic diversity indices (see

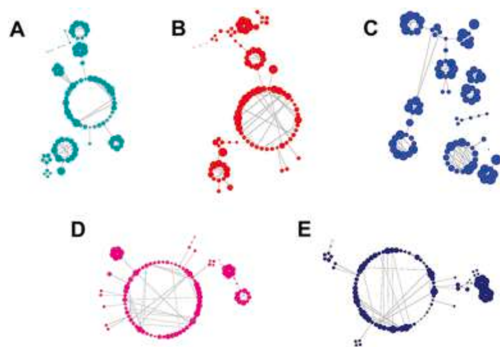

Fig. 5. Fine-scale population genetic structure networks of individual NRS *O. niloticus* var. GIFT broodstock groups and reference strains generated using Netview R. Networks A (cyan) and B (red) represent NRS future brooder ponds 1 and 2 respectively, while network C (blue) represents NRS mass production pond NP-8. Networks D (magenta) and E (navy blue) display the WorldFish GIFT 9th generation and ASL 11th generation reference strains respectively. Each sample group was analysed separately, with networks generated at mk-NN = 5 for each sample group and laid out in a circular topology framework. Node size for each network is mapped to relatedness of individual fish. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2). As expected, the GIFT reference strain followed by ASL individuals showed the lowest degree of relatedness to all NRS fish. However, NRS RP-4 samples were slightly more unrelated ($r = -0.0221 \pm 0.0047$) to the reference GIFT animals, than either TG-4 ($r = 0.0013 \pm 0.0019$) or NP-8 ($r = -0.0014 \pm 0.0018$) individuals. Among the NRS sample groups, RP-4 fish showed lower relatedness to both other ponds; $r = 0.0015 \pm 0.0112$ and 0.0035 ± 0.0116 for NP-8 and TG-4, respectively. Similarly, intra-pond relatedness was much lower for RP-4 samples ($r = 0.0147 \pm 0.0194$) compared to both other ponds; TG-4 $r = 0.0580 \pm 0.0097$ and NP-8 $r = 0.0592 \pm 0.0123$. These data suggest that wild *O. niloticus* may have mixed with GIFT fish in pond RP-4 during flooding events at NRS.

4. Discussion

The objectives of this study were to determine the genetic status of the GIFT strain of *O. niloticus* broodstock maintained at NRS, and to assess the viability of these fish for continued fry/fingerling production in Fiji. Results show that genetic diversity in the NRS broodstock has declined moderately since their introduction 20 years ago, compared to ninth-generation GIFT and ASL reference sample groups. Uncontrolled reproduction and slight inbreeding are also evident, however the broodstock nucleus does not appear to have suffered a major loss of genetic diversity since introduction to Fiji. Analyses of genetic structure

Fig. 6. Admixture barplots generated for individual NRS *O. niloticus* var. GIFT broodstock groups and reference strains using the ADMIXTURE analysis package (Alexander et al., 2009). Each plot was constructed at individual k-thresholds of 3 through 6, respectively. Ancestry proportions are represented on the vertical axes, while population sample groups are ordered along the horizontal axes, respectively. NRS pond labels FB 1, FB 2 and MP correspond with ponds TG-4, RP-4 and NP-8 respectively.

Table 4
Summary of COLONY results for *O. niloticus* var. GIFT broodstock groups and reference strains. The numbers of full-sib and half-sib dyads, combined number of full-sib and half-sib dyads, best (Maximum Likelihood) full-sib families and best clusters are reported after ordering against a probability threshold of p ≤ 0.01. The number of best full-sib families is divided into families containing 2 or more members, and singletons (sole members). Numbers of putative parents are reported only from the best clusters (p ≤ 0.01).

Relatedness measure	NRS FB 1 pond TG-4 (*n* = 93)	NRS FB 2 pond RP-4 (n = 94)	NRS MP pond NP-8 (n = 93)	All NRS samples (n = 280)	WorldFish GIFT reference (n = 94)	ASL reference (n = 94)
Number of full-sib dyads (p ≤ 0.01)	65	21	102	523	34	66
Number of half-sib dyads (p ≤ 0.01)	148	118	91	973	57	26
Number of combined full-sib and half-sib dyads (p ≤ 0.01)	213	139	193	1496	91	92
Number of best (ML) full-sib families identified (p for inclusion ≤0.01)	48	78	42	120	61	53
The numbers of families with 2 or more members/ singleton members identified (p for inclusion ≤0.01)	19/29	9/69	15/27	40/80	21/40	20/33
Number of best (ML) clusters identified (p ≤ 0.01)	55	18	47	228	49	31
Number of putative mothers identified within best (ML) clusters	13	13	15	54	23	12
Number of putative fathers identified within best (ML) clusters	22	14	12	43	26	12

and relatedness also revealed admixture of founding individuals, likely due to a combination of stock management practices and past flooding events that NRS has experienced. Very little genetic differentiation has occurred in the intervening 20 years since introduction of the GIFT line to Fiji, and as a result the broodstock nucleus has become analogous to a wild, natural population retaining much of its genetic diversity, due to minimal management interventions.

While these findings indicate that the NRS broodstock nucleus has retained much of the genetic diversity accumulated during the GIFT selective breeding programme, it remains to be seen whether its culture performance remains comparable with other GIFT lines. A comprehensive evaluation of culture performance of the NRS GIFT fish should provide insights into whether the valuable, high performing alleles acquired during generation of the GIFT line are still present, or if they have been lost as a result of founder effects and broodstock management practices. This study has also demonstrated the utility of genome-wide SNPs for characterisation of wild and farmed tilapia resources, and may also be applied to other aquaculture commodities for breeding and rearing management.

4.1. Broodstock management and fingerling production practices

The management strategy for the broodstock pool of GIFT fish maintained at NRS is minimalistic, with very limited interventions to control age and size structuring, as well as relatedness. The future brooder pool appears only to be replenished with fry and fingerlings produced by cohorts sourced from within the future brooder pool itself, which is likely to result in successive generations of closed breeding.

The system in place for routine fry and fingerling production is complex, and completely reliant on the availability of high-quality broodstock from the future brooder pool. The architecture of this production system has arisen in response to strong demand from farmers across the country for GIFT fingerlings, and therefore the breeding management goals of NRS have had to adapt to both produce and distribute large quantities of GIFT seed, and concurrently maintain broodstock quality. These two objectives are discordant in practice, as management actions for maintaining genetic quality of a broodstock pool often conflict with actions required for mass fingerling production. Implementation of the mass fry production system (e.g. pond NP-8) is an example of the latter, where, in the short term, high numbers of fry and fingerlings can be produced, but at the expense of seed quality due to inbreeding depression over the longer term.

Due to the current, (and likely future) limited availability of resources and personnel at NRS, the tasks of maintaining the genetic integrity of broodstock and performing stock improvement activities in parallel with routine seed production, are not feasible. Therefore, we propose three strategies for addressing the issues of broodstock management and fry/fingerling production at NRS. These include 1) segregation of the broodstock nucleus to control age and size structuring, 2) monitoring of genetic diversity and relatedness within the nucleus and 3) transitioning towards more efficient fry production technology, such as artificial egg incubation (Bhujel, 2009; Bhujel, 2011). Infrastructure improvement at NRS is also vital, to mitigate pond inundations during flood events, so that undesired mixture of separate broodstock pools does not occur.

4.2. Genetic diversity, inbreeding and relatedness of current GIFT broodstock

Maintenance of high levels of genetic diversity is imperative for the long-term success of selective breeding and seed production of any cultured species, as it ensures that captive populations possess adaptive capacity to environmental stressors or changes, and also retain sufficient phenotypic variability to enable targeted selection (Wada and Jerry, 2008). For many species used in aquaculture, routine production and management practices are capable of significantly eroding standing levels of genetic diversity, due to population founder effects, small effective broodstock numbers, grading and selection effects, as well as differential family contributions and survival rates (Durand et al., 1993; Frost et al., 2007; Wada and Jerry, 2008).

Loss of genetic diversity in closed, captive populations is usually observed when cultured stocks are produced from small founding broodstock numbers, and when individuals are subjected to several generations of closed breeding (Frost et al., 2007; Wada and Jerry,

Table 5

Pairwise relatedness (*r*) estimates computed for the *O. niloticus* GIFT populations sampled using the triadic likelihood (TrioML) estimator in the COANCESTRY software package. 95% confidence interval limits following bootstrapping (n = 1000) are reported in parentheses below the estimated *r* values.

	NRS future brooder pond TG-4	NRS future brooder pond RP-4	NRS mass production pond NP-8	WorldFish GIFT reference	Abbassa Selection Line reference
NRS future brooder pond TG-4	0.0580 (±0.0097)				
NRS future brooder pond RP-4	0.0035 (±0.0116)	0.0147 (±0.0194)			
NRS mass production pond NP-8	0.0519 (±0.0093)	0.0015 (±0.0112)	0.0592 (±0.0123)		
WorldFish GIFT reference	0.0013 (±0.0019)	−0.0221 (±0.0047)	−0.0014 (±0.0018)	0.0999 (±0.0064)	
Abbassa Selection Line reference	−0.0887 (±0.0009)	−0.0677 (±0.0024)	−0.0862 (±0.0008)	−0.0625 (±0.0008)	0.2624 (±0.0061)

2008). This is especially relevant to the establishment of breeding nuclei using GIFT fish, as the progenitors of these individuals will already have been subjected to several generations of targeted selection for desirable traits (Bentsen et al., 2016; Eknath and Acosta, 1998; Eknath et al., 1993). Consequently, great care must be taken in the management of these stocks to retain the superior culture performance characteristics of the GIFT line.

During the current evaluation of the GIFT breeding nucleus at NRS, it is evident that genetic diversity has declined to a small extent in all broodstock sample groups, with reduced effective population sizes and the magnitude of inbreeding varying between ponds, in comparison to both reference strains. Despite these observations, this loss of diversity has not been severe, as there are no signatures of inbreeding depression or excess homozygosity. Perhaps somewhat counter intuitively, the holding of broodstock in 8 separate ponds may have assisted in retention of diversity over a large pool of individuals, together with rotation of broodstock through the hatchery system. Over the 20 years since introduction, losses of diversity in the broodstock nucleus may be restricted to rare alleles, as a result of population founder effects, random genetic drift and stock management practices.

Diversity measures and the genetic structure of broodstock sampled from ponds TG-4 and NP-8 were comparable to the GIFT reference strain, while fish sampled from pond RP-4 displayed different trends. These included a low effective population size, increased private allelic richness and lower heterozygosity, as well as signatures of genetic sub-structuring. These patterns may have arisen as a result of wild *O. niloticus* entering the pond during flooding events, as shown in relatedness estimates, and appear as divergent individuals and families. Examined collectively, it is also clear that all broodstock held at NRS have become admixed since their introduction, and with high levels of relatedness across all ponds sampled, they now constitute a single large population.

These findings are contradictory to results reported by McKinna et al. (2010), where reduced genetic diversity was detected among NRS broodstock when compared to reference samples of sixth generation WorldFish GIFT fish. These authors mention that inbreeding rates appeared to be on the rise, and that deterioration of culture performance observed at the time may be correlated with the loss of valuable GIFT alleles. It is possible that limited marker resolution (1 mtDNA locus and 4 microsatellite loci), and/or the number of individuals sampled (n = 29) could have contributed to these findings.

Evaluation of the genetic diversity of the GIFT line introduced in other locations has revealed more optimistic results than those reported here. In Malaysia for example, mate allocation strategies employed for sixth generation GIFT families post-introduction in 2001/2002, has resulted in minimised inbreeding rates and a satisfactory effective population size to sustain a selective breeding programme (Ponzoni et al., 2005; Ponzoni et al., 2009). However, Ponzoni et al. (2010) cautioned that while the effective population size of 88 determined for this nucleus is adequate for containing inbreeding and maintaining heritability, it is still below the recommended threshold of 500 for maintaining evolutionary potential (Franklin and Frankham, 1998). Similarly in Sri Lanka, heterozygosity has been maintained and inbreeding minimised in three GIFT lines from generations six and nine, suggesting they are competent for further use (De Silva, 2015). Reduced genetic diversity has been documented in breeding systems for other species used in aquaculture, including barramundi (Frost et al., 2007), rainbow trout (D'Ambrosio et al., 2019) and pearl oysters (Wada and Jerry, 2008), underscoring the need for careful broodstock selection and monitoring.

4.3. Current NRS GIFT germplasm viability

Conservation of the genetic integrity of a broodstock population relies on maximising genetic variation, and minimising inbreeding, while retaining the heritabilities of valuable culture traits (Fernández et al., 2014). For Nile tilapia breeding programmes, this poses a particular challenge owing to the biological characteristics of the species, which include short generation intervals, low reproductive output and early sexual maturity (Hussain, 2004; Lind et al., 2015), making broodstock nuclei susceptible to inbreeding. While a certain amount of inbreeding can be tolerated in animal breeding programs (approximately 1% per generation, Franklin, 1980), rapid inbreeding can very quickly lead to inbreeding depression and associated negative stock fitness consequences (Wada and Jerry, 2008).

On the basis of data generated during the current study, while very little genetic diversity has been lost and levels of genetic differentiation are low in the NRS GIFT broodstock pool, it is unclear whether the remaining genetic diversity is sufficient to maintain the nucleus over the long term. It is therefore important to determine if valuable GIFT alleles have been retained or possibly lost since introduction of the GIFT line to Fiji, by assessing the culture performance of the nucleus. The results of this exercise would determine if the genetic potential of the NRS GIFT fish has been preserved, and outline future strategies for its maintenance. Additionally, because stock in pond RP-4 appear to be admixed with germplasm of unknown origin, breeding from these animals should be avoided and the fish held separately, until testing is carried out to ascertain their relatedness to the NRS GIFT pool.

4.4. Implications for management

Over the short term, improved stock management practices are required for management of the Fijian GIFT genetic resource, to ensure that further erosion of valuable genetic diversity is minimised. Over the longer term, a thorough evaluation of culture performance is recommended, with accompanying genetic monitoring of the broodstock nucleus at regular intervals.

4.4.1. Culture performance evaluation of NRS GIFT broodstock

Reports of negative stock performance, including but not limited to early sexual maturity, depressed growth rates and reduced harvest weights and sizes provided the impetus for the current study, and also

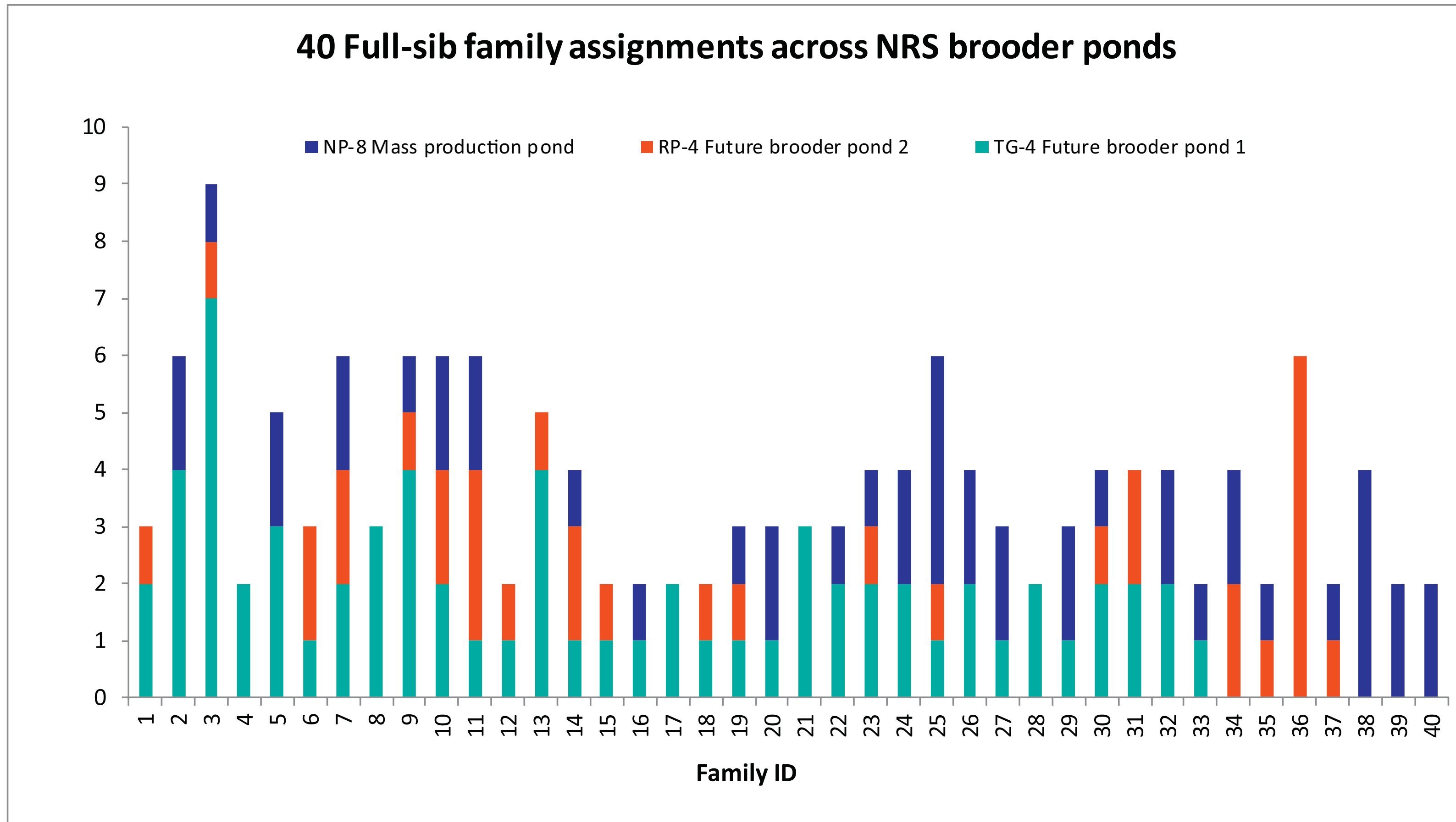

Fig. 7. Distribution of 40 full-sib family assignments containing two or more members only generated by COLONY, across ponds sampled for all NRS *O. niloticus* var. GIFT broodstock.

for the work carried out by McKinna et al. (2010). In the light of the results reported here, the genetic integrity of the NRS GIFT broodstock nucleus has largely been retained, albeit with a minor loss of genetic diversity. In order to conclusively determine if the reported culture performance deterioration in farmed fish is related to the loss of genetic diversity measured and described here, the culture performance of the NRS broodstock nucleus requires formal evaluation.

A study for assessment of culture performance could adopt the methods of Macaranas et al. (1997), Neves et al. (2008) and Mwanja et al. (2016). The former authors evaluated the reproductive, growth and survival performance of four tilapia strains at NRS over three generations, comparing *O. mossambicus*, *O. niloticus* var. 'Israel', *O. niloticus* var. 'Chitralada', and a red tilapia hybrid strain (*O. mossambicus* × *O. niloticus*). Results showed that *O. niloticus* var. 'Chitralada' following a weighted performance assessment across all traits, was the best performing strain under Fijian conditions. A culture performance trial could also identify the highest performing families present within the NRS GIFT nucleus, and these could be tracked for retention, while underperforming families may be pruned from the nucleus.

Phenotypic signatures of inbreeding depression known for Nile tilapia include reduced reproductive success (Fessehaye et al., 2009), reduced survival and physical abnormalities and deformities (Ponzoni et al., 2010). If the results of the culture performance assessment indicate that growth and survival rates are depressed, or other signatures of reduced diversity are evident, this could suggest that desirable GIFT alleles may have been lost. Conversely however, if culture performance is as expected (see Bentsen et al., 2016; Eknath and Acosta, 1998; Eknath et al., 1993; Ponzoni et al., 2010), then other culture inputs such as husbandry and feeds or feeding management regimes may be examined to determine causative factors behind the impaired performance observed.

4.4.2. Maintenance and genetic monitoring of the breeding nucleus

The current practice at NRS of mass spawning broodstock, especially in the mass fingerling production system and in the knockdown tanks to an extent, can result in highly uneven parental contributions to subsequent generations, resulting in lower effective population sizes. Management interventions over and above current practices are required to mitigate this effect, by spawning broodstock in smaller groups following size segregation, and ensuring that where possible, each brooder contributes only once to the next generation (Bentsen et al., 2016; Ponzoni et al., 2010). Maintenance of pedigree records are also critical in this respect, to ensure stock traceability for mate allocation.

5. Conclusion

Using a genome-wide approach, high-resolution data on the genetic diversity, inbreeding and relatedness of Fijian GIFT *O. niloticus* broodstock has been generated, and used to assess the viability of the NRS GIFT germplasm for seed production. The data indicate that declines in genetic diversity have been dectectable, however signatures of inbreeding depression and strong genetic differentiation are absent. Consequently, the broodstock nucleus is likely to be suitable for continued use in fry and fingerling production for supply to tilapia farmers; however, an evaluation of stock performance is required to determine if any valuable GIFT alleles have been lost, which would manifest in impaired culture productivity.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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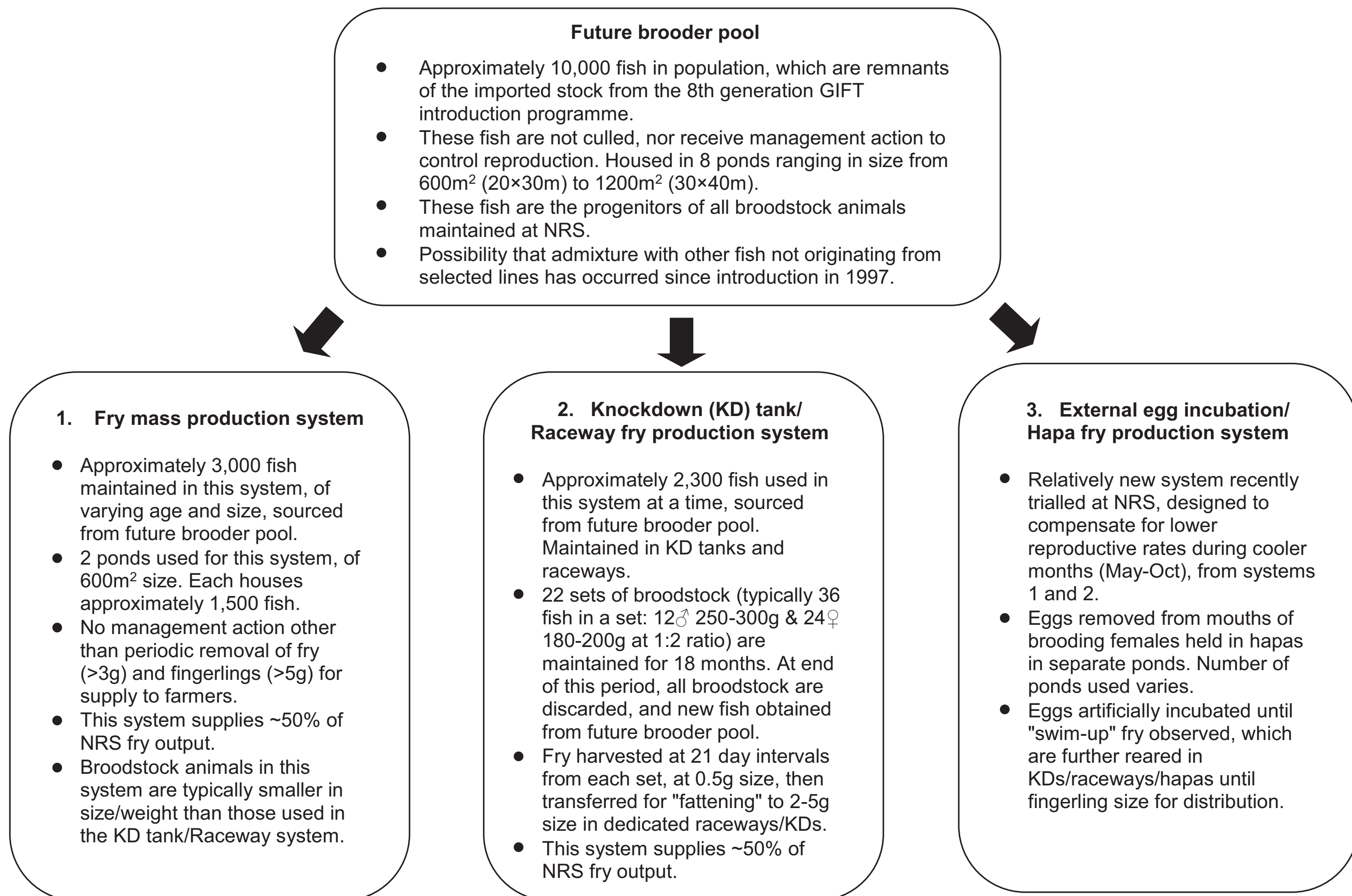
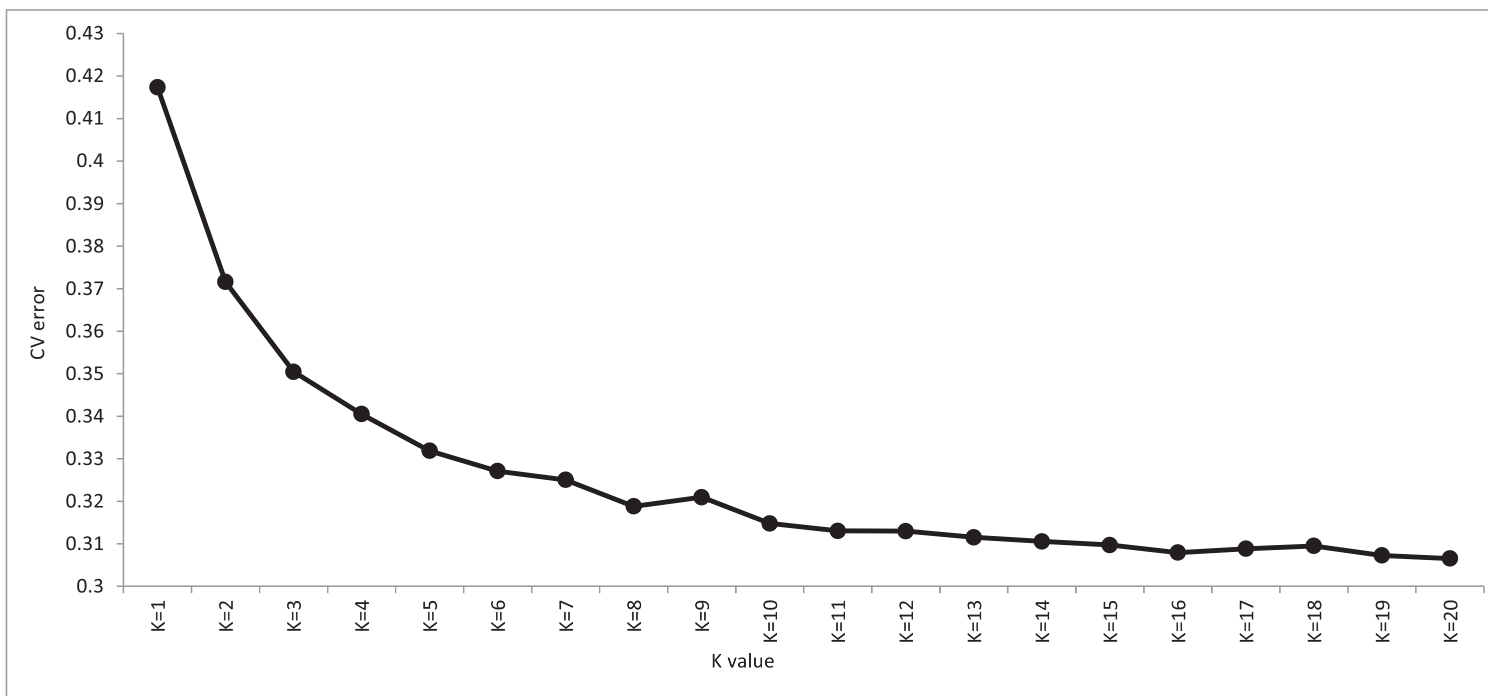
Appendix A. Current tilapia fry production system at NRS in July 2017



Appendix B. ADMIXTURE analysis cross-validation statistic plot for assessment of an optimal k-threshold. K-values specified for individual runs are represented on the horizontal axis, while the cross-validation error values generated for each run are represented on the vertical axis



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