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Genetic parameters and response to selection for body weight, fillet traits, body size traits and survival in Nile Tilapia (*Orechromis niloticus*)

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Master programme in Aquaculture

Genetic parameters and response to selection for body weight, fillet traits, body size traits and survival in Nile Tilapia (*Oreochromis niloticus*)

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Abstract

This study evaluates the genetic parameters and selection responses in relation to harvest body weight, fillet yield, fillet weight, body size traits (length, depth, thickness) and survival of GenoMar Supreme Tilapia (GST™) strain (*Oreochromis niloticus)*, which is currently at the 25th generation and the main continuation of the Genetic Improvement of Farmed Tilapias (GIFT) program. The breeding scheme implemented by GenoMar relies on DNA-marker-based tagging of the fish, and does not require the families to be grown separately until they reach a certain size. In this breeding scheme, all breeding candidates are reared in a common pond and eventually nearly 4% of them are pre-selected based on growth. Fillet yield (fillet weight*100/body weight) records and survival information are obtained from the full sibs of the breeding candidates, which are reared in a separate pond. The animals are selected for improved growth, fillet yield and survival. The body size traits (length, depth, thickness) are also measured, but not selected for.

In this study, pedigree starting from the base generation of GIFT (3rd generation) and phenotypes starting from the 12th generation were used. The overall heritabilities (h^2) of the body weight, fillet yield, and survival were 0.179 ± 0.024, 0.215 ± 0.023, and 0.183 ± 0.024 respectively. Full-sib effect explained nearly 10% and 6% of the variation in body weight and fillet yield, respectively. The genetic correlation between body weight and fillet weight was large (0.861±0.002), suggesting that increased body weight may lead to increased fillet weight. Likewise, the genetic correlations between body weight and body size traits were high and positive. Fillet yield was genetically correlated with body weight positively and in a moderate magnitude (0.395±0.003), which indicates that increased body weight may result in improved fillet yield of the fish. Survival was positively correlated with all other traits. The overall selection responses for body weight (from 14th generation), fillet yield (from 17th generation), and survival (from 21st generation) were 0.19, 0.16, and 0.07 genetic standard deviations, respectively. The results suggest that the breeding program was responsive to selection and there is room for improvement in the future generations.

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1. Introduction

Tilapias are known for their tolerance of and adaptability to a wide range of farming systems, making them indispensable for many fish farmers with limited resources (Bentsen et al. 1998; Eknath et al. 1993). The production of tilapia exceeded 5.6 million tons in 2015, ranking it the second most produced fish species worldwide, with most of the production coming from the developing countries in Asia. Among all the tilapias farmed, Nile tilapia (*Oreochromis niloticus*) is the most preferred one by farmers; making up more than 65% of the total production (3.9 million tons) of the tilapia farming industry (FAO 2017).

However, the tilapias farmed in Asian aquaculture systems back in the 1980s exhibited genetic founder and bottleneck effects, genetic deterioration, and inbreeding due to poor management practices (Acosta & Gupta 2010; Pullin & Capili 1988), thereby, disqualifying them from any further genetic improvement by selective breeding. Therefore, the need to establish and manage a well-documented base population that has a broad genetic variation was apparent. To address this, "Genetic Improvement of Farmed Tilapias" (GIFT) project started in 1988 with the intention of bringing new germplasm of *O. niloticus* and establishing a base population through applying the latest selective breeding technology (Bentsen et al. 1998; Bentsen et al. 2017). The GIFT project, aiming to supply the fish farming industry with the genetically improved stocks, was terminated in 1997 after successfully satisfying its main goals, proving that selective breeding was a robust method to improve the tropical finfish genetically (Acosta & Gupta 2010). At this stage, the selection had been carried out for 10 generations. Earlier generations of the strain had then been used to establish the genetic source of other breeding programs in several countries (Ponzoni et al. 2010). However, the GenoMar Supreme Tilapia™ (GST) strain, which is carried out by GenoMar AS, is the main continuation of the original GIFT strain, and the only one that had access to a full copy of the 10^{th} generation.

The main trait of interest for selection in the GIFT program was growth performance due to its high economic importance to the farmers (Bentsen et al. 2017). However, fillet traits (fillet weight and fillet yield) of tilapia have gained importance in recent years because the customers in the main export countries (e.g. Europe and the US) prefer to buy fillets instead of the whole fish (Fitzsimmons et al. 2011; Thodesen et al. 2012). Therefore, the tilapia in

the GST selective breeding program are being selected for improved growth performance and fillet yield. The GST breeding program also includes the survival trait, which is of crucial importance to increase the profitability (Chiayvareesajja et al. 1999).

GenoMar applies a novel breeding scheme that relies on DNA marker based tagging of the fish to utilize the large number of full sibs available, as described by Skaarud et al. (2014). In this breeding scheme, all breeding candidates are reared in a common pond and eventually nearly 4% of them are pre-selected based on growth. Only the pre-selected individuals are DNA typed and then PIT tagged. To obtain an even number of fillet yield records and survival information from all families, an "informant group" is established, which consists of full-sibs of the breeding candidates. These full-sibs are slaughtered to obtain fillet yield information and thus are not eligible as selection candidates for the next generation. This family selection scheme requires rearing large numbers of families, and sufficient number of individuals within each family to obtain accurate information. A downside with the family selection method is that it exploits solely between family genetic variation and not within family variation (Haffray et al. 2013). Several researchers have investigated whether using body measurements (body weight, length, depth, thickness) is a viable method to predict the fillet weight and fillet yield (Gjerde et al. 2012; Nguyen et al. 2010; Rutten, M. J. et al. 2005) and they concluded that fillet weight can be relatively accurately predicted from body measurements because of the strong correlations between body measurements and fillet weight. However, fillet yield is always predicted with significantly lower accuracy than fillet weight when using body measurements.

Considering the GIFT program as the beginning, GST selective breeding program has been ongoing for 25 generations to date. A previous study investigated the genetic gain for and relationship between body weight and fillet yield using data from 17 generations (Yalew 2007); however, no reports are available that use data from all the 25 generations. Therefore, the objectives of this thesis are to estimate i-) the genetic and phenotypic parameters for body weight and fillet traits along with body size traits (length, depth, thickness) and survival, and ii-) the obtained selection response for body weight, fillet traits, and survival using 25 generations of the GST program.

2. Materials and methods

2.1. Base population

The base population of the GIFT project is the base population of the GST program as well, since the latter is a continuation of the former. The work to establish the base population started with collecting four wild African (from Egypt, Ghana, Kenya, and Senegal) and four farmed Asian strains (from Israel, Singapore, Taiwan, and Thailand). Subsequently, as first-generation trials, all the collected strains were tested in eight different farm environments. This was followed by the second-generation trials, in which a complete diallel crossing experiment (8x8 strains) was conducted. A base population with broad genetic variation was then established by using the best performing strains. More information about the base population of the GIFT project can be found in Eknath et al. (1993). Selection for improved growth started in the base generation, which is named as the 3rd generation in this study.

2.2. Production of families

2.2.1. Mating of breeders

GenoMar handles each generation of fish in eight, almost monthly, batches. Splitting each generation into batches distributes the workload evenly throughout the whole year and helps to utilize the facilities in a more efficient way. A new batch is produced only after all the work with the current batch is completed. In each batch, typically 600 DNA-typed (300 males and 300 females) individuals are available for selection.

All 600 breeders are first stocked in concrete tanks and later transferred to earthen ponds for conditioning where the breeding nucleus of GST is located, the Philippines, city of Muñoz in Central Luzon. Brood stock feeds are given daily for 1-2 weeks. After conditioning of the breeders, the best 30 males and 30 females are selected. These selected individuals with the highest breeding values are mated; however, care is taken to avoid the mating of closely related individuals. Contribution of the selected breeders are limited to 5 males/females from the same full-sib family. A revolving breeding scheme is followed in which, females from batch number "*n"* are mated with the males from batch number "*n+1"*

(i.e. younger sires are mated with older dams). A visualisation of the pedigree structure was established using the "pedantics" package in R software (Morrissey & Wilson 2010). The batch mating method is utilized to improve the breeding success and reduce the risk of inbreeding. "Ready to spawn" females whose genital papillae is swollen and pink/red in colour, are given priority in mating. The mating takes place in breeding hapas in which one male is mated with one female (pair-wise mating). When mouth brooding is observed after mating, the feeding stops and the fry is collected after 7-10 days of stocking the breeders. The inbreeding coefficients of each individual were calculated using the "pedigree" package in R software (Coster & Coster 2010).

2.2.2. Establishment of nursery hapas

In each batch, 20-35 families are produced. Breeding is carried out in 1-2 weeks. If the minimum number of required families is not reached within 2 weeks, another week may be used. After the families are produced, the ones that have low survival rates (see section 2.5) are discarded. The families with few individuals are also discarded except for the ones with high average breeding values. However, if there are more than 30 families available, the families with few individuals are discarded completely. The produced fry is transferred to three different nursery hapas, which are mass selection (MS), full sib fillet yield (FS FY), and full sib back up (FS BU). Up to 15000 – 18000 individuals (1-600 fry/family) are transferred to MS hapas. The FS FY and FS BU groups consist 800-1200 individuals (25-40 fry/family) and 1000-1500 individuals (1-50 fry/family), respectively. In all nursery hapas, the stocking density is 150 fish per meter square. FS FY fish is sex reversed by hormone treatment (starting from the 17^{th} generation), resulting in all males, apart from a few female individuals that existed in some batches after sex reversion.

2.2.3. Rearing of families and tagging

For the pre-grow-out phase, all families of MS and FS FY are transferred to 2000 m^2 and 500 m^2 common ponds, respectively. All families of FS BU fish; on the other hand, are transferred to a common tank at a backup location. The fish is fed daily and feeding regime is corrected by sampling the weight of the fish every month. Sexing of the MS fish is done at 6^{th} week, and the males and females are reared separately. At the same time, all the

female fish are removed from the FS FY group. The pre-selection of the animals in the MS groups are carried out at 16th week, and 3000 biggest individuals (1500 males and 1500 females) are pre-selected. The fish that are not selected are kept for a month to serve as backup in case of mortalities occur in the selected fish. Final harvesting of the fish is conducted at 30th week and the biggest 600 individuals (300 males and 300 females) are selected from MS groups. All the selected fish is PIT tagged and fin clipped after conditioning for at least 3 days in tanks. The fish from FS FY group; however, is only fin clipped.

Out of 600 available breeders, the best 60 (30 males and 30 females) are selected according to selection index (see section 2.3) and production of the next generation starts as described in the section 2.2.1.

2.3. Traits recorded

At harvest, the body weight (BW) of all 600 pre-selected breeders from the MS group, along with all the individuals from the FS FY group is recorded (to the nearest 0.1 g). The fish were grown to about 250 g of weight until the 16th generation. Starting from the 17th generation, the fish were grown heavier and bigger (700 – 750 g) to measure further growth and fillet yield. The pre-selected individuals from the MS group are the ones that will be used in mating, thus it is not possible to obtain fillet yield records on them. Instead, the full sibs (FS FY) of the pre-selected individuals are filleted and, fillet yields (FY) and fillet weights (FW) are recorded. Fillet yield were started to be measured regularly on the FS FY animals in the 17th generation, which is the generation that selection for increased fillet yield began. Before the 17th generation, only body weight measurements were taken in the FS groups. Five skilled people did the filleting of the fish. Fillets are skinned but not trimmed. Fillet yield is calculated by dividing the fillet weight by body weight and multiplying the result with a hundred (FY= [FW/BW] *100). In addition to fillet yield, survival data is obtained from the FS FY group, as well. Survival (S) was recorded as family values. Survival value of a family represents the proportion of its members that survived the grow out phase. Typically, there are 40 fish in each full sib family at the beginning and survivors are counted after the grow-out phase. The proportion of the survivors is calculated by dividing the number of survivors by the total number of individuals present in the

beginning. Thus, each family has a single survival value. Selection for increased survival started in the 21st generation even though the measurements of survival were made available starting from the 19th generation. The body size traits, which are standard length (L), depth (D), and thickness (T) were measured on both MS and FS FY groups using callipers (to the nearest 0.1 mm). Depth and thickness were measured at the mid part of the fish, where they were thickest. Length was measured as the distance from the tip of the mouth to the tip of caudal peduncle of the fish. The body size measurements were started to be taken in the latest batch of the 14th generation. The breeders are selected for increased harvest body weight, fillet yield, and survival, which constitute the selection index. The relative weights of the traits in the selection index changed through the generations (Table 2-1). Body size traits (length, depth, thickness), on the other hand, are not selected for and recorded only to observe how the breeding values for these traits change and calculate the genetic correlations with other traits recorded.

Table 2-1.The relative weights of the traits in different spans

	Body Weight	**Fillet Yield**	**Survival**
Until Generation 17	100%	-	-
Generation 17 Batch 1 - Generation 20 Batch 6	50%	50%	-
Generation 20 Batch 7 - Generation 21 Batch 4	30%	30%	40%
Generation 21 Batch 5 - Generation 25 Batch 8	40%	40%	20%

2.4. Statistical models for body weight, fillet measurements, and body size measurements

The variance components of body weight, fillet measurements (FY and FW), and body size measurements (L, D, and T) were obtained by restricted maximum likelihood fitting univariate mixed animal models both within generations and across generations using ASReml version 4.1 (Gilmour et al. 2015). In matrix notation, the single trait animal model may be written as

$$y = X\beta + Zu + Wc + e$$

where $\mathbf{y}$ is a vector of standardized body weight, fillet weight or body size measurements, or observed fillet yield, $\boldsymbol{\beta}$ is the vector of fixed effects of sex and batch (filleter identity is included only in the analysis of fillet measurements), $\mathbf{u}$ is the vector of random animal additive genetic effects $\sim (0, \mathbf{A}\sigma_a^2)$ where $\mathbf{A}$ is the additive genetic (numerator) relationship matrix among all the animals in all generations, $\mathbf{c}$ is the vector of effects common to full sibs other than additive genetic effect $\sim (0, \mathbf{I}\sigma_c^2)$, and $\mathbf{e}$ is the vector of random residual effects $\sim (0, \mathbf{I}\sigma_e^2)$. $\mathbf{X}$, $\mathbf{Z}$ and $\mathbf{W}$ are known incidence matrices, that relate observations to the fixed effects, the additive genetic effect of the individual animal and the common full sib effect included in the model, respectively. The expectations of $\text{cov}(\mathbf{u},\mathbf{e})$ and $\text{cov}(\mathbf{u},\mathbf{c})$ are zero and $var\ (y) = \boldsymbol{ZGZ`} + \boldsymbol{WIW`} + \boldsymbol{R}$. The genetic correlations among the traits were calculated as the correlations among the estimated breeding values (EBV).

Only individuals from the FS FY groups were used in the statistical analyses since the FS FY fish are a random, and relatively equal sample from all families. MS fish, on the other hand, are not a random sample of all families since only the largest pre-selected individuals are recorded. Thus, the data of the MS fish were not used in the statistical analyses. The phenotypes of the individuals before the 10th generation, that is before the transition took place, were not used. The phenotypes of the individuals in the 10th and 11th generations were also not used due to the reasons explained in section 2.6. The pedigree however, started from the base generation (3rd generation).

Heterogeneity of phenotypic variances across FS FY batches for traits body weight, fillet weight and body size measurements were accounted for by standardisation of the observations to a common variance of 1 by dividing each observation by the standard deviation of its batch. After running the analyses with the standardized phenotypes, all the outliers reported by ASReml were removed from the data (except for body weight) and the analyses were run one more time with the outliers removed data sets. For the analysis of body weight, 12th, 13th and 25th generations, and all the batches except for 5th and 7th in the 18th generation were removed completely since these generations and batches had a zero or close to zero heritability (see section 3.4) and reduced the overall heritability of body weight. After running the model for body weight with this reduced data set, all outliers reported by ASReml were also removed. Removing the outlier phenotypes increased the

heritability estimates in general. Editing of the data (see supplementary materials) were performed using R Software version 3.3.2 (R Core Team 2016; Wickham & Francois 2015; Wickham 2016). The outliers reported by ASReml corresponded to the data which was 3.7 – 6.71 absolute standard deviation away from the mean for body weight (a total of 141 outliers), 3.7 – 12.04 absolute standard deviation away from the mean for fillet yield (a total of 420 outliers), 3.7 – 5.95 absolute standard deviation away from the mean of fillet weight (a total of 118 outliers), 3.7 – 21.55 absolute standard deviation away from the mean of length (a total of 378 outliers), 3.7 – 19.22 absolute standard deviation away from the mean of depth (a total of 283 outliers), 3.7 – 12.64 absolute standard deviation away from the mean of thickness (a total of 303 outliers). The recordings of the traits are prone to human error, which might be a major reason that extreme phenotypes existed in the dataset. The extreme observations that are below the overall mean might also occur due to sickness, in which case the animals do not represent the genetic value of their families, and thus it is hardly of any value to include these animals in the genetic analyses. Therefore, the estimated breeding values of animals and the genetic correlations among the traits were calculated using the outliers removed data sets.

The effect common to full sibs includes the maternal environmental effect and one quarter of the dominance effect, which are completely confounded in the present data. Since all the animals from each family are DNA tagged and raised in one single environment, there is no common environmental effect due to separate rearing of the families.

The heritability for body weight, fillet measurements, and body size measurements were calculated as $h^2 = \frac{\hat{\sigma}_a^2}{\hat{\sigma}_a^2+\hat{\sigma}_c^2+\hat{\sigma}_e^2}$ and the common full sib effect as $c^2 = \frac{\hat{\sigma}_c^2}{\hat{\sigma}_a^2+\hat{\sigma}_c^2+\hat{\sigma}_e^2}$ where $\hat{\sigma}_a^2$ is the additive genetic variance, $\hat{\sigma}_c^2$ is the variance common to full-sibs, and $\hat{\sigma}_e^2$ is the error variance. The genetic correlation between the traits measured was calculated as $r_g = \frac{\hat{\sigma}_{a_1 a_2}}{\sqrt{\hat{\sigma}_{a_1}^2}\sqrt{\hat{\sigma}_{a_2}^2}}$ where a_1 and a_2 are the estimated breeding values (EBV) of the same individuals for two different traits.

2.5. Statistical model for survival

The survival values in this study, as described in section 2.3, are the mean survival rates of families. Thus, a mixed model that uses family mean survival rates as the trait was fitted as described by Lin (2016). A total of 1459 family means were used. The model fitted may be expressed as

$$y = X\beta + (Z_s + Z_d)u + e$$

where **y** is the vector of mean survival rates per family, **β** is the vector of fixed effect batch, **u** is the vector of random additive genetic effects of sires and dams, and **e** is the vector of random residual effects. X, Z_S, Z_d are known incidence matrices that relate observations to the fixed effect, random sire effect, and random dam effect, respectively.

The heritability for survival was calculated as $h^2 = \frac{4\hat{\sigma}_u^2}{4\hat{\sigma}_u^2 + \hat{\sigma}_e^2}$, where $\hat{\sigma}_u^2 = \hat{\sigma}_s^2 = \hat{\sigma}_d^2 = 1/4\hat{\sigma}_a^2$ in which $\hat{\sigma}_s^2$ is the additive genetic sire variance and $\hat{\sigma}_d^2$ is the additive genetic dam variance. The full-sib effect was not included in the model to achieve convergence. The genetic correlations of survival with other traits measured were calculated in the same way as described in section 2.4.

2.6. Response to selection

The responses to selection were calculated as genetic standard deviation differences between the generations.

During the transition period from GIFT to GST (10th generation), the fish were kept at the maintenance level and not allowed to grow, which is referred as "stunning". The fish were kept until they were too old and several fish died. Therefore, the 11th generation was established with the survived individuals and no selection for higher performance was implemented. Upon the completion of the transition, the selection for increased body weight restarted. Lingering unfavourable effects of the stunning; however, were still apparent in the subsequent generations. For this reason, a relaxed selection protocol was applied until the 14th generation. The fish were started to be selected intensively beginning from the 14th generation, therefore, the selection response for body weight was calculated starting from the 14th generation.

During the rearing and harvesting of the 22nd generation, problems with parasitic infestations were experienced. Parasites and frequent treatment caused deformities and mortalities on the fish, which prevented the mating of the individuals with the highest breeding values to establish the next generation. Instead, the individuals with lower breeding values were used in reproduction, which caused a reduction in the average breeding value of the next generation for body weight (see section 3.6).

The relative weight of growth reduced in the selection index as fillet yield started to be incorporated in the breeding objectives (starting from the 17th generation).

The selection response for body weight was therefore calculated once for the span of 14th – 17th, once for 17th – 22nd and once for the span of 22nd – 25th generations. Further calculations were made for the spans of 14th – 22nd and 14th – 25th generations. The indirect selection responses for body size traits were calculated for the same spans, as well.

The selection responses for fillet yield and survival were calculated starting from the 17th and 21st generations, respectively.

3. Results

3.1. Pedigree structure and inbreeding

The pedigree structure of the GST is shown in Figure 3-1. The triangle shapes in the figure represent the batches that were mated with each other.

There were no new animals introduced into the breeding program. Some individuals in the pedigree structure appear to be not related to former generations, since they are unassigned because of tag losses.

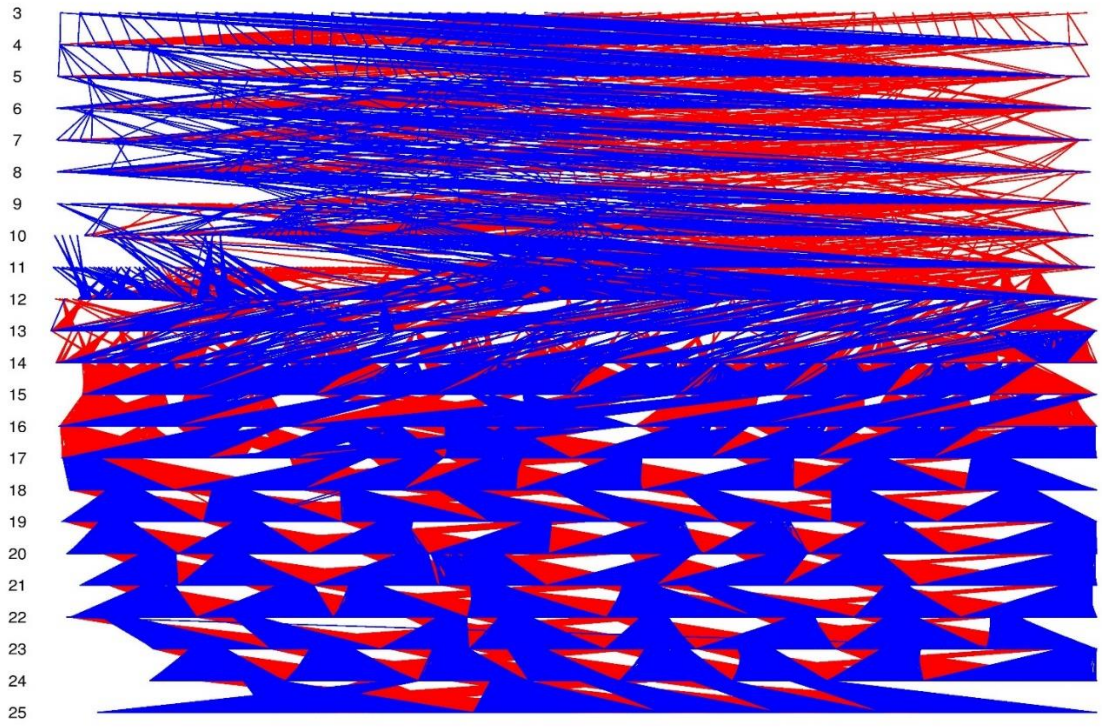


Figure 3-1.The pedigree structure of GST. X axis represents the generations. Paternal contributions were represented in blue and maternal contributions are represented in red. The triangle shapes represent the batches.

The change of the average inbreeding coefficients is shown in Figure 3-2. The accumulated coefficient of inbreeding was 7.1% in the 25th generation. After the transition from GIFT to GenoMar, the average increase in the coefficient of inbreeding was 0.3% per generation.

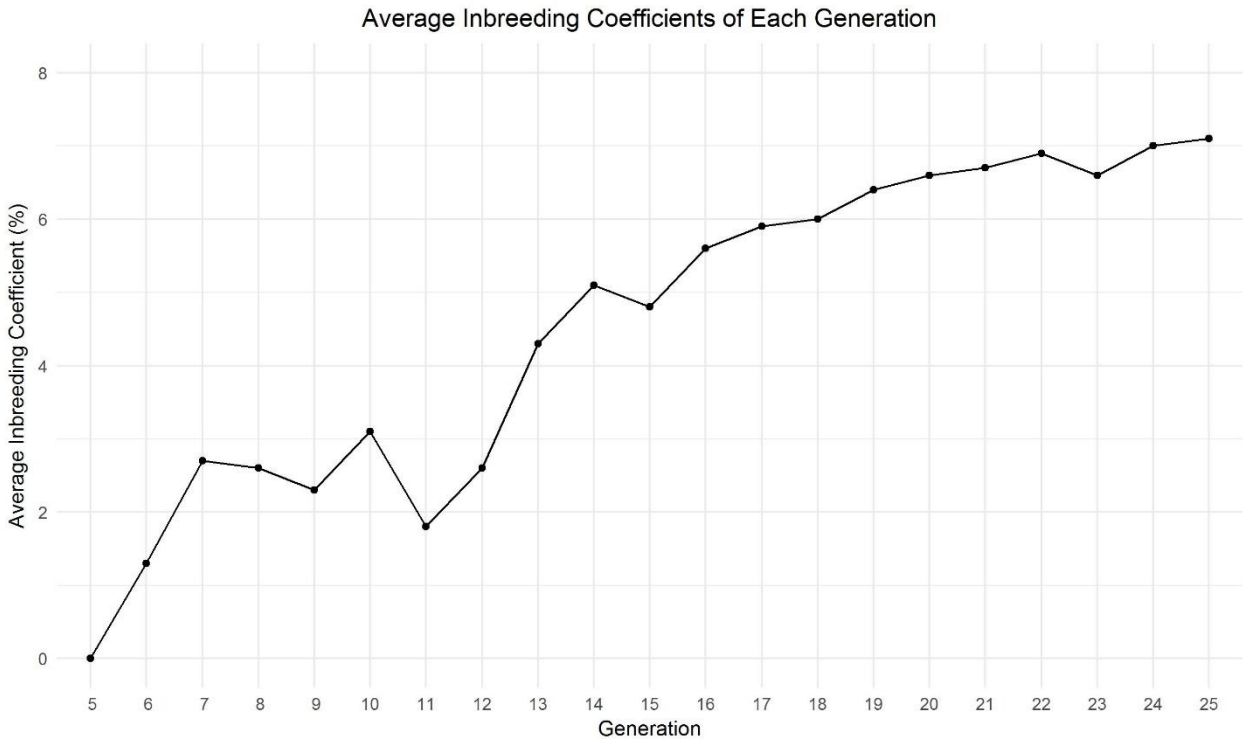


Figure 3-2. The change of the average coefficient of inbreeding of each generation. The average inbreeding coefficient was zero in the 3rd, 4th, and 5th generations.

3.2. Descriptive statistics

Descriptive statistics for the studied traits (except for survival) and the number of individuals of FS FY group for each generation before removing any outliers are shown in Table 3-1.

The coefficient of variation (CV) of body weight varied among the generations. The highest and lowest CVs were 44.7% (14th generation) and 16.3% (13th generation). However, the CV of body weight did not vary much among the generations other than 13 and 14. The overall CV of body weight was 49.6%; however, it was 32% when the span of 17th – 25th generations was considered. The males were on average 38.3% heavier than females ($p<0.001$) when generations 12-16 (in which the fish was not sex reversed) were taken into account.

Table 3-1. The number of individuals (n) in each generation, means and coefficient of variations (CV %) of all traits measured

Generation	N	Mean BW	CV BW	Mean FY	CV FY	Mean FW	CV FW	Mean Length	CV Length	Mean Depth	CV Depth	Mean Thickness	CV Thickness
12	2501	201.1	25.3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
13	3567	250.7	16.3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
14	3696	301.0	44.7	NA	NA	NA	NA	25.0	7.3	11.4	31.6	5.4	10.5
15	2320	252.5	33.4	NA	NA	NA	NA	17.9	10.1	8.0	13.7	4.3	10.1
16	1800	247.0	29.9	NA	NA	NA	NA	18.0	7.8	7.8	11.0	NA	NA
17	3252	630.6	25.7	38.3	8.2	242.8	28.3	24.1	9.0	11.1	9.6	35.4	39.2
18	3744	563.4	27.4	38.0	7.0	220.2	27.5	23.0	8.9	10.6	10.4	40.8	9.6
19	4120	687.5	26.3	38.0	6.5	262.4	28.8	24.6	7.8	11.2	9.7	43.8	7.7
20	3492	677.6	27.0	37.8	7.2	257.9	29.2	24.6	9.5	11.1	11.9	44.1	8.5
21	4261	713.0	21.7	37.5	7.1	268.9	24.7	25.2	8.3	11.4	8.9	45.9	6.5
22	5052	787.3	32.2	37.8	6.5	298.9	34.4	26.0	10.3	11.6	11.7	48.3	11.9
23	2291	765.0	37.9	37.3	7.5	287.6	40.2	25.5	12.5	11.5	14.7	52.4	13.2
24	2542	728.2	37.9	37.0	7.3	271.5	40.7	25.5	11.4	11.2	15.7	49.8	15.3
25	3831	725.3	34.9	37.1	7.0	271.5	37.8	25.8	10.3	11.5	12.9	48.5	10.3
Overall	46469	565.9	49.6	37.6	7.6	262.7	34.6	24.2	13.7	11.0	15.7	44.2	22.8

BW= Body Weight, FY= Fillet Yield, FW= Fillet Weight

Average fillet yield ranged between 37.0% (24th generation) and 38.3% (17th generation). The CVs for fillet yield were low and ranged between 6.5% (19th and 22nd generation) and 8.2% (17th generation). Fillet yield of males was 1.1% higher than females ($p<0.01$).

Descriptive statistics for survival as family means are shown in Table 3-2. The highest mean survival value was observed in 22nd generation (56%) and the lowest was observed in 23rd generation (23%). The CVs for survival were high and varied from 31.3% to as high as 85.4%.

Table 3-2. Number of families (n) per generation, mean survival rates of families and coefficient of variations (CV %)

Generation	n	Mean Survival	CV Survival
19	236	0.45	40.1
20	162	0.47	41.4
21	209	0.43	58.9
22	219	0.56	31.3
23	193	0.23	69.4
24	220	0.26	85.4
25	220	0.42	58.9
Overall	1459	0.40	58.0

Descriptive statistics of each trait after the outlier phenotypes removed can be found in Supplementary material 1.

3.3. Fixed effects

Body weight, fillet and body size measurements were significantly influenced by sex and batch effects. Fillet measurements were also significantly influenced by the filleter identity. Survival was significantly influenced by batch, which was the only fixed effect included in the analysis of the survival trait (Table 3-3).

Table 3-3. The significance levels of the fixed effects for each trait

Fixed Effect	BW	FY	FW	L	D	T	S
Sex	***	**	***	***	***	***	-
Batch	***	***	***	***	***	***	***
Filleter	-	***	***	-	-	-	-

BW= Body Weight, FY= Fillet Yield, FW= Fillet Weight, L= Length, D= Depth, T= Thickness, S= Survival

** $p<0.01$

*** $p<0.001$

3.4. Genetic parameters

The estimates for heritabilities and common environmental effects are shown in Table 3-4.

Table 3-4. Heritability and full-sib effects for all the traits studied. "Full Data" refers to the analyses in which all the phenotypes were included. "Outliers Removed Data" refers to the analyses in which the outlier phenotypes were taken out.

	Full Data		Outliers Removed Data	
Trait	**h^2**	**c^2**	**h^2**	**c^2**
BW	0.132 ± 0.018	0.113 ± 0.007	0.179 ± 0.024	0.101 ± 0.009
FY	0.156 ± 0.019	0.039 ± 0.006	0.215 ± 0.023	0.058 ± 0.008
FW	0.121 ± 0.020	0.092 ± 0.008	0.122 ± 0.020	0.093 ± 0.008
L	0.199 ± 0.022	0.073 ± 0.007	0.272 ± 0.025	0.082 ± 0.008
D	0.122 ± 0.019	0.098 ± 0.008	0.148 ± 0.021	0.119 ± 0.008
T	0.111 ± 0.019	0.067 ± 0.007	0.141 ± 0.021	0.076 ± 0.008
S	0.183 ± 0.024	-	-	-

BW= Body Weight, FY= Fillet Yield, FW= Fillet Weight, L= Length, D= Depth, T= Thickness, S= Survival

The overall h^2 estimate of body weight was low in magnitude (0.132); however, the h^2 estimates of single generations fluctuated greatly, varying between 0.000 and 0.436 (Table 3-5). The generations that had the lowest h^2 values (12^{th}, 13^{th}, 25^{th} generations) and six of the batches in the 18^{th} generation (batches other than 5 and 7) were dropped from the overall estimates of heritability. Generations 12 and 13 were likely to be influenced by the stunning of the fish in the previous generations, which prevented the animals to grow normally and masked their genetic potential. 18^{th} and 25^{th} generations were affected either because of management or disease problems. Furthermore, all the outliers reported by ASReml were removed. The overall h^2 estimate of this clean data was 0.179, which was a 36% increase. The overall c^2 has reduced from 0.113 (full data) to 0.101 (clean data).

Table 3-5. Heritability and full-sib effects of separate generations for body weight and fillet yield

	Body Weight		Fillet Yield	
Generation	**h^2**	**c^2**	**h^2**	**c^2**
12	0.000 ± 0.000	0.291 ± 0.032	-	-
13	0.018 ± 0.068	0.090 ± 0.033	-	-
14	0.193 ± 0.089	0.011 ± 0.032	-	-
15	0.246 ± 0.094	0.002 ± 0.030	-	-
16	0.137 ± 0.103	0.073 ± 0.044	-	-
17	0.436 ± 0.117	0.007 ± 0.042	0.208 ± 0.035	0.000 ± 0.000
18	0.047 ± 0.074	0.141 ± 0.037	0.170 ± 0.080	0.042 ± 0.032
19	0.214 ± 0.107	0.120 ± 0.043	0.172 ± 0.076	0.031 ± 0.029
20	0.300 ± 0.120	0.062 ± 0.046	0.146 ± 0.083	0.040 ± 0.034
21	0.099 ± 0.073	0.058 ± 0.031	0.020 ± 0.068	0.094 ± 0.032
22	0.189 ± 0.089	0.069 ± 0.036	0.223 ± 0.030	0.000 ± 0.000
23	0.116 ± 0.082	0.044 ± 0.033	0.215 ± 0.046	0.000 ± 0.000
24	0.432 ± 0.053	0.000 ± 0.000	0.132 ± 0.093	0.080 ± 0.041
25	0.000 ± 0.000	0.136 ± 0.036	0.022 ± 0.061	0.085 ± 0.029

The overall h^2 estimate of fillet yield was low in magnitude (0.156) and h^2 estimates of single generations were relatively stable, except for the drops in the 21st and 25th generations (Table 3-5). The c^2 accounted for 3.9% of the phenotypic variance of fillet yield. Removing the outliers from the data changed the h^2 estimate to a medium magnitude (0.215), which was a 38% increase. The c^2 accounted for 5.8% of the phenotypic variance after removing the outliers.

The overall h^2 estimate of survival was low in magnitude (0.183). The c^2 effect was not estimated because of convergence problems.

The overall h^2 estimates of fillet weight, length, depth, and thickness were all low in magnitude and 0.121, 0.199, 0.122, and 0.111, respectively. All the heritability estimates increased after removing the outlier phenotypes for each trait. The changes in the full-sib effects can be seen in Table 3-5.

3.5. Genetic and phenotypic correlations

The genetic and phenotypic correlations among all the traits studied are shown in Table 3-6.

The genetic correlation between body weight and fillet weight (0.861), as well as between body weight and body size traits (0.835 – 0.887) was very high, indicating that selection for increased body weight would lead to improved fillet weight and increased body sizes. The genetic correlation between fillet yield and body weight (0.395), on the other hand, was moderate in magnitude, which indicates that the genetic gain for fillet yield would not be as high as the genetic gain for fillet weight if selection is based on increased body weight.

Survival trait was genetically correlated with body weight in moderate magnitude (0.37), which suggests that survival would be improved if selection based only on improved growth.

The phenotypic correlations were generally of similar magnitude as the genetic correlations. The phenotypic correlation between body weight and fillet yield (0.04); however, was notably lower than the genetic correlation of the respective traits. Furthermore, the phenotypic correlation between fillet yield and survival was negative (-0.06) even though the genetic correlation of the respective traits were positive.

Table 3-6. Genetic (below the diagonal) and phenotypic (above the diagonal) correlations among the traits and associated standard errors

	BW	FY	FW	L	D	T	S
BW		0.040 ± 0.005	0.942 ± 0.002	0.875 ± 0.003	0.835 ± 0.003	0.602 ± 0.004	0.427 ± 0.024*
FY	0.395 ± 0.003		0.218 ± 0.005	0.004 ± 0.005	0.044 ± 0.005	0.079 ± 0.005	-0.060 ± 0.026*
FW	0.861 ± 0.002	0.607 ± 0.002		0.780 ± 0.003	0.757 ± 0.004	0.592 ± 0.004	0.441 ± 0.024*
L	0.840 ± 0.002	0.299 ± 0.002	0.760 ± 0.002		0.842 ± 0.002	0.624 ± 0.004	0.411 ± 0.024*
D	0.887 ± 0.001	0.346 ± 0.001	0.838 ± 0.002	0.771 ± 0.002		0.660 ± 0.004	0.401 ± 0.024*
T	0.835 ± 0.002	0.523 ± 0.002	0.905 ± 0.001	0.696 ± 0.002	0.798 ± 0.002		0.416 ± 0.024*
S	0.37 ± 0.02*	0.36 ± 0.02*	0.36 ± 0.02*	0.32 ± 0.02*	0.36 ± 0.02*	0.34 ± 0.02*	

BW= Body Weight, FY= Fillet Yield, FW= Fillet Weight, L= Length, D= Depth, T= Thickness, S= Survival

* based on family averages

3.6. Response to selection

The genetic gains in standard deviation differences for each trait for different spans are shown in Table 3-7.

Table 3-7. The genetic gains for each trait in genetic standard deviation differences for different spans of generations

	Gen 14-17	**Gen 17-22**	**Gen 22-25**	**Gen 14-22**	**Gen 14-25**
Body Weight	0.27	0.22	0.11	0.21	0.17
Fillet Yield	-	0.16	0.20	-	-
Fillet Weight	-	0.24	0.16	-	-
Length	0.21	0.2	0.09	0.18	0.15
Depth	0.22	0.22	0.13	0.19	0.17
Thickness	0.07	0.23	0.12	0.17	0.15
Survival	-	-	0.12	-	-

The selection response for body weight for the span of 14^{th} – 17^{th} generations (0.27) was higher than other spans of generations (0.11 and 0.22), since the harvest body weight was the only trait selected for during the 14^{th} – 17^{th} generations. When selection is performed for only increased body weight, the genetic gain for length and depth in genetic standard deviations were similar to that of body weight. The selection responses for body weight and body size traits were approximately the same for the span of 14^{th} – 25^{th} generations.

The change of average estimated breeding values (EBV) for harvest body weight is shown in Figure 3-3.

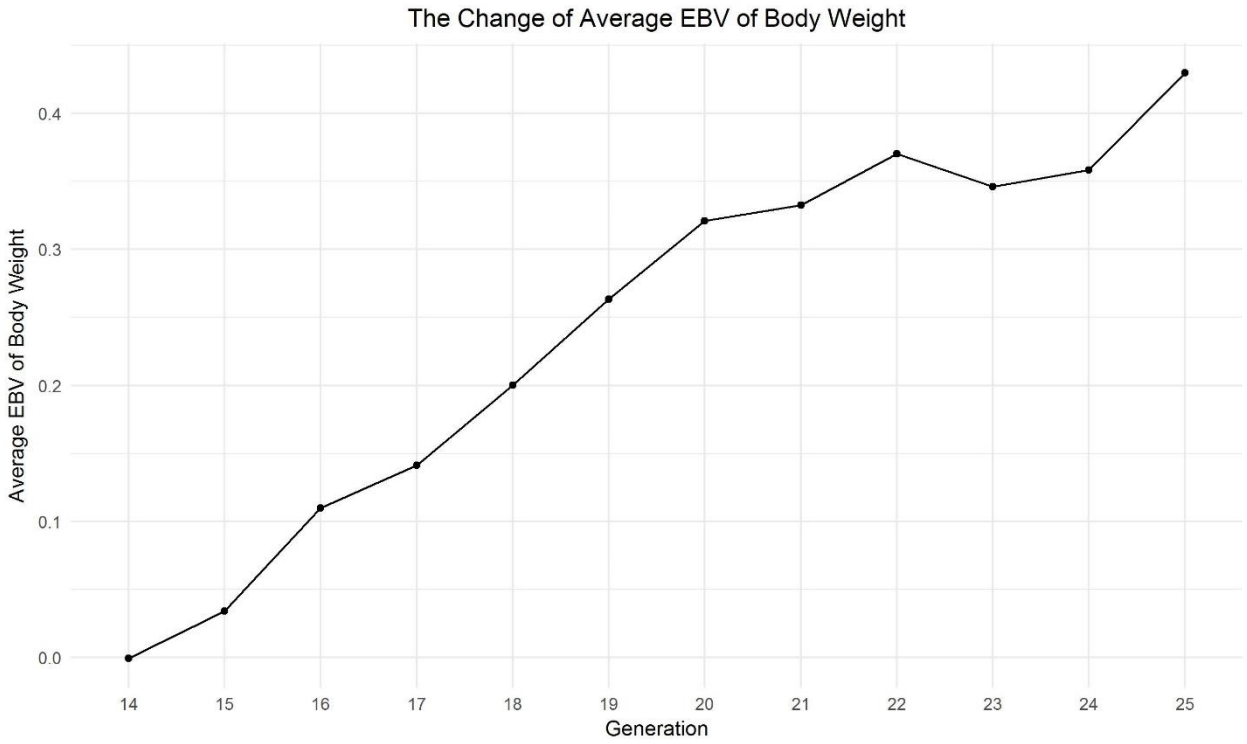


Figure 3-3. The change of average estimated breeding values of body weight for each generation. The phenotypes of 12th, 13th, and 25th generations were excluded from the data for the calculation of genetic gain, since these generations had very low heritabilities due to the reasons given in the text.There is a steady increase in the average EBVs of body weight until the 22nd generation, which is followed by a reduction in the 23rd generation (the reason for this reduction was explained in section 2.6). The selection responses for body weight for the span of 14th – 22nd and 14th – 25th generations were 0.21 and 0.17 genetic standard deviation, respectively.

The selection response for fillet yield for the span of 17th – 25th generations (in which generations the selection for increased fillet yield was applied) was 0.17 genetic standard deviation (Figure 3-4). The selection response for fillet weight for the span of 22nd – 25th generations was 0.20 genetic standard deviation.

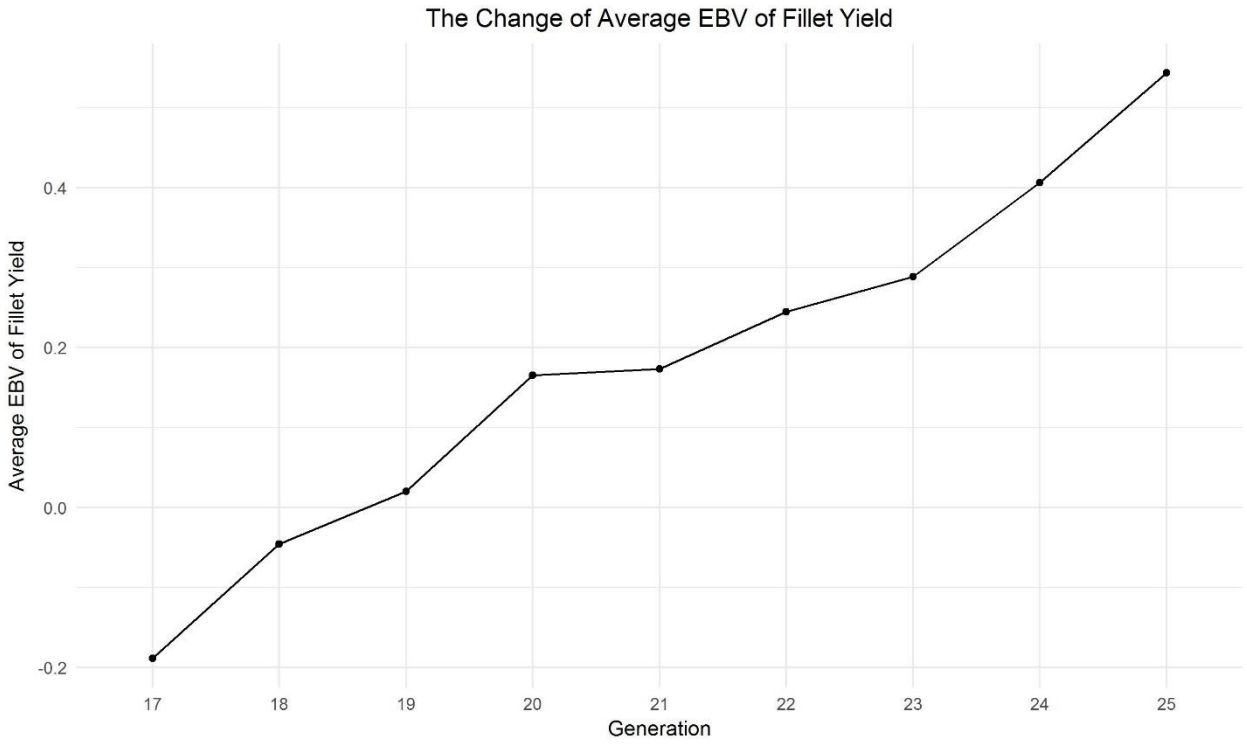


Figure 3-4. The change of average estimated breeding values of fillet yield for each generation

The selection response for survival for the span of 21st – 25th generations (in which generations the selection for increased survival was applied) was 0.07 genetic standard deviations (Figure 3-5). The response for survival was higher for the span of 22nd – 25th generations (0.12), where parasitic infestations and mortalities occurred. The selection response for survival was not always positive in every generation. Average EBVs for survival reduce in the 22nd and 24th generations

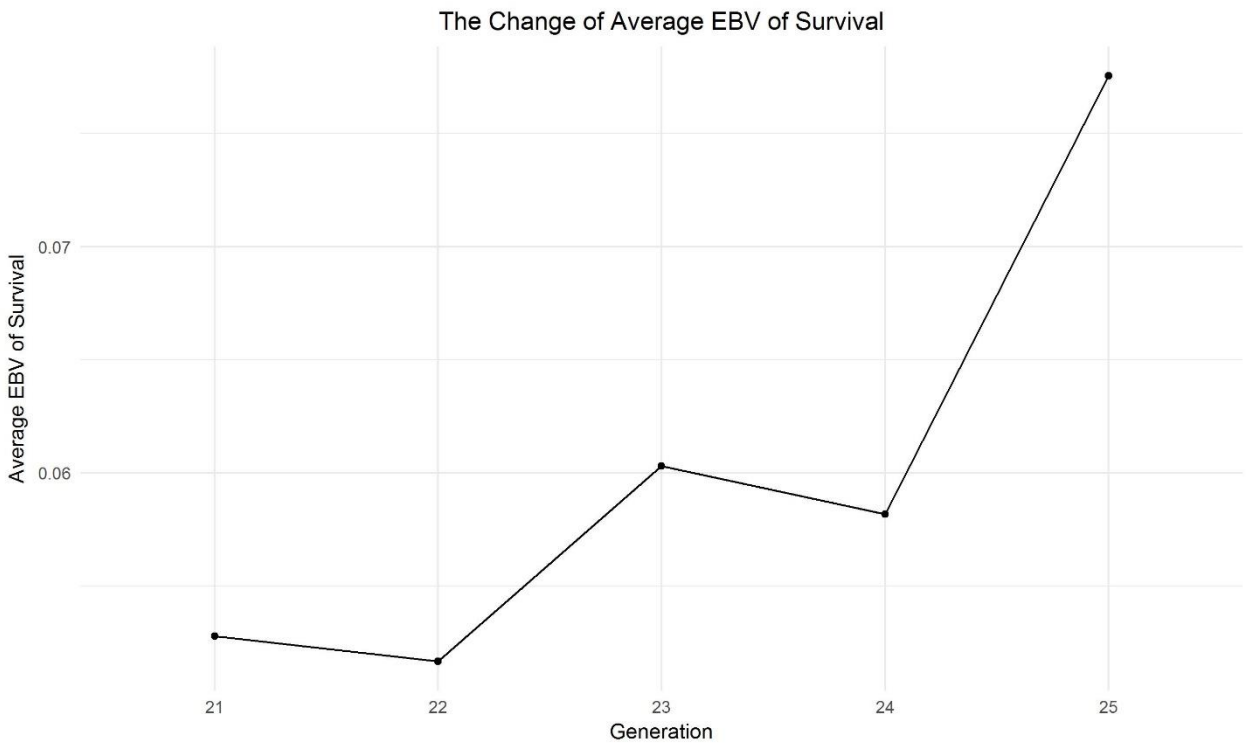


Figure 3-5. The change of average estimated breeding values of survival for each generation

4. Discussion

4.1. The selective breeding program

In this study, the selective breeding program of GenoMar Supreme Tilapia, which, to the best of my knowledge, is the longest running aquatic breeding program regarding the number of generations produced, was examined. The fish is currently at the 25th generation and the improvement program has undergone 22 generations of selection for increased body weight, 8 generations of selection for improved fillet yield, and 4 generations of selection for increased survival. There is no reduction in the variance of body weight, fillet yield, and survival. Parameters estimated for the traits under selection indicate that there is still genetic variation in the GST strain, and there is still room for further selection and performance improvement.

GenoMar introduced two novelties to the GIFT project after the transition took place, which are the DNA marker based tagging system and batch mating method. DNA tagging of the animals removes the need to rear the families separately until the fish is big enough to be

physically tagged. Furthermore, DNA tagging helps to reduce the generation interval and increase the selection intensity, which leads to faster genetic gain (Gjøen 2004).

4.2. Body weight

The overall response to selection for body weight was 0.17 genetic standard deviations, which indicates that the current breeding program functions adequately for improving the growth trait. The highest selection response for body weight was observed in the span of 14^{th} – 17^{th} generations (0.27 genetic standard deviation), in which the body weight was the only trait that was selected for. The response for body weight was lower (0.22 genetic standard deviations) when fillet yield started to be included in the breeding objectives (17^{th} – 22^{nd} generations). The reduction in the average EBVs of animals in the 23^{rd} generation that was caused by parasitic infestations and mortalities illustrates the fact that animals should be managed well and kept away from diseases to conduct an effective breeding program. The selection response for body weight was 0.11 genetic standard deviation for the span of 22^{nd} – 25^{th} generations, which was the lowest response of all three spans.

The overall coefficient of variation for body weight at harvest was around 50%, which was higher than the values reported by Gjerde et al. (2012); Marjanovic et al. (2016); Nguyen et al. (2010); Ponzoni et al. (2005); Rutten, M. J. et al. (2005); Thodesen et al. (2011); Trọng et al. (2013) but lower than the value reported by Nguyen et al. (2007). As described in the section 2.3, the fish were grown to different pre-defined harvest weights in different generations, which might be the main factor that causes an overall CV of around 50%. The highest CV for body weight was observed in the 14^{th} generation, which is possibly related to the stunning and relaxed selection in the previous generations. The CVs of body weight did not vary greatly among generations after the 14^{th} generation, which might be related to the fact that average increase of coefficient of inbreeding per generation was low.

The significant difference between the harvest body weights of males and females found in this study seems to justify the commercial production of only male tilapia.

The estimated heritability for harvest body weight of 0.179 ± 0.024 was lower than the values estimated by de Oliveira et al. (2016); Hamzah et al. (2014); Marjanovic et al. (2016); Rutten, M. J. et al. (2005); Zak et al. (2014), similar to the value estimated by

Gjerde et al. (2012), and higher than the values estimated by Bolivar and Newkirk (2002); Brzeski and Doyle (1995). The animals in this study were DNA tagged and raised in a single environment, thus the full-sib effect was made up by only maternal environmental effect and one quarter of the dominance effect. Therefore, a low full-sib effect was expected; however, the full-sib effect for body weight was large (0.101 ± 0.009) and similar to the values found in the experiments where physical tagging methods were used (Bolivar & Newkirk 2002; Charo-Karisa et al. 2006; Thodesen et al. 2011; Zak et al. 2014). This could be explained by that the maternal effects, such as egg size and quality have a very important effect in tilapia and compose the most prominent part of the full-sib effect. The heritability of body weight varied among the generations. The varying heritability among the generations is most likely due to the factors other than genetic ones, such as changing weather conditions, diseases, and varying water quality.

4.3. Fillet traits

Fillet is the most valuable part of fish, of which high quantities are both desired and demanded by the industry. Fillet weight is expected to increase as the weight of fish increases. This was also the case in this study where fillet weight was strongly correlated with body weight (0.861 ± 0.002). However, what is more desirable is to obtain heavier fillet without further increasing the body weight i.e. body weight does not change but fillet becomes heavier, which can be only achieved through selecting for improved fillet yield. This also enables that more of the fish is exploited, and therefore creates more value per fish. Some authors discuss that direct selection for ratio traits, like fillet yield, is a challenging task due to statistical hurdles (de Oliveira et al. 2016; Gjerde et al. 2012). For example, if the coefficients of variation of two traits (e.g. body weight and fillet weight in this study) are vastly different, the ratio trait may not be responsive to selection. However, the coefficients of variation of body weight and fillet weight in the current study were similar in generations 17 and further. In addition, response to selection for a ratio trait is undermined as the ratio gets closer to either 0 or 100%. The average fillet yield found in this study (37.6%) was at an intermediate level, and therefore was regarded as responsive to direct selection. Furthermore, the heritability of fillet yield was far from zero. Consequently, the improvement of fillet yield by direct selection was deemed possible in

the GST population. The positive genetic correlation between fillet yield and body weight indicates that increased body weight would lead to improved fillet yield; however, including fillet yield in the breeding objectives is necessary to achieve a satisfactory selection response. However, the selection should not focus only on fillet yield. A farmer's primary interest is generally the total output of the farm. Thus, body weight or fillet weight should be incorporated in the breeding objectives along with fillet yield (Rutten, M. J. et al. 2005).

The selection for increased fillet yield in the current breeding program appear to be effective as the selection response was calculated as 0.17 genetic standard deviation for the span of 17th – 25th generations. The genetic improvement of fillet yield does not seem to be affected by the parasitic infestations that affected the improvement of body weight as the selection response was 0.20 genetic standard deviation for the span 22^{nd} – 25^{th} generations. The selection response for fillet yield for the span of 20^{th} – 21^{st} generations was 0.02 genetic standard deviation, which illustrates the fact that, to obtain a meaningful selection response, the weight of the trait fillet yield in the selection index should be more than 30%.

In this study, the fillet was skinless but not trimmed. With this fact in mind, the average fillet yield of 37.55% ± 2.32% was similar to the values reported by Gjerde et al. (2012); Nguyen et al. (2010); Turra et al. (2012) and higher than the values reported by Clement and Lovell (1994); Rutten, M. J. et al. (2005); Rutten et al. (2004). The overall CV for fillet yield (7.6%) found in this study was higher than the values reported by Rutten, M. J. et al. (2005); Rutten, M. J. M. et al. (2005), and lower than the value reported Nguyen et al. (2010). A high average fillet yield with a considerably high CV may be the result of the low increase of coefficient of inbreeding per generation achieved in the studied population.

The estimated heritability of 0.215 ± 0.023 for fillet yield was higher than the values reported by Gjerde et al. (2012); Rutten, M. J. et al. (2005) but lower than the value reported by Nguyen et al. (2010). In addition, the heritability of fillet yield was relatively stable among the generations, which suggests that fillet yield is not as much sensitive to environmental changes as body weight. This is also supported by the relatively low overall full-sib effect estimated for fillet yield (0.058 ± 0.008).

4.4. Body size traits

The genetic correlations between the body size traits and body weight, as well as among body size traits were very high, which is an indication that these traits are influenced by the same set of genes (Nguyen et al. 2010). The body size traits were genetically correlated to body weight and fillet weight in stronger magnitude than they were genetically correlated to fillet yield. Very high genetic correlations between body weight and body size traits support the discussion of Nguyen et al. (2007), who concluded that body size measurements can be used as a selection criterion to obtain an indirect selection response for body weight when it is not possible to record harvest weight. The highest indirect selection responses were obtained for length and depth when the animals were selected for only increased body weight (generations 14-17). When the selection was performed for both increased body weight and improved fillet yield, the highest indirect selection response was obtained for thickness. Therefore, selection for thickness alone can be an effective indirect selection criterion for increased body weight and fillet yield, when it is not possible to obtain measurements of body weight and fillet yield.

4.5. Survival

The selection for improved survival started in the 21^{st} generation and it seems to be effective as the overall selection response was 0.07 genetic standard deviations for the span of 21^{st} - 25^{th} generations. The selection response for survival was 0.1 genetic standard deviation for the span of 22^{nd} – 23^{th} generations, which is an indication that the mortalities occurred due to parasitic infestations in the 22^{nd} generation acted favourably for the genetic gain of the survival trait. The reductions in the average EBVs in the 22^{nd} and 24^{th} generations are most likely caused by specific disease problems experienced in the preceding generations. In other generations, the cause of mortalities was probably more general reasons, which leads to increased response for survival. If a specific pathogen causes most of the mortalities, the selection should focus on improved resistance for that specific pathogen. However, if there is not a dominant pathogen that harms the animals in a population (as is the case for the GST strain), the selection for improved survival would be adequate.

The estimated heritability of 0.183 ± 0.024 for survival was low in magnitude and higher than the value estimated by Thodesen et al. (2013) for blue tilapia, lower than the value estimated by Luan et al. (2008) and similar to the value estimated by Rezk et al. (2009). The fact that different diseases and factors cause mortalities in different populations may be an explanation for the differences of the heritabilities of different populations. Survival rate varying to a great extent among different generations (23 – 56%) seems to have been influenced by environmental factors like changing weather conditions, water quality, and diseases. Therefore, improving the environmental conditions and management practices is imperative to achieve high survival rates.

It is possible to obtain a positive genetic gain for survival by selecting for increased body weight and fillet yield; however, indirect genetic gain for survival would be low since the genetic correlations among the respective traits were not high. Thus, the trait survival must be incorporated in the breeding objectives of tilapia although the heritability of survival was found to be low in magnitude, considering the crucial importance of a high survival rate of the fish for the profitability of fish farmers.

4.6. Inbreeding

The average increase of the coefficient of inbreeding was 0.3% per generation, which indicates that the batch mating method was effective in keeping the inbreeding low. Low average increase of the coefficient of inbreeding per generation also suggests that even stricter selection intensity can be achieved to improve the selection responses.

The accumulated coefficient of inbreeding in the 25^{th} generations was found to be 7.1%. Bentsen et al. (2017) reported an accumulated coefficient of inbreeding of 7.1% for the 5^{th} generation of GIFT. The pedigree used in this study consisted only the individuals who contributed to the later generations, and no information was available for the 1^{st} and 2^{nd} generations. The pedigree used by Bentsen et al. (2017) most likely consisted all the individuals, not only the ones who contributed to the later generations, which might be the reason of the difference of the coefficients of inbreeding found in the two studies.

5. Conclusion

Results indicate that the selective breeding program of the GST™ is effective as there were considerable genetic gains for growth, fillet yield, and survival. The positive genetic correlations among the traits indicates that selection for increased body weight would result in indirect genetic improvement in fillet yield and survival; however, including fillet yield and survival in the breeding objectives would lead to higher genetic gains for these traits. The genetic correlations between the body size traits and other traits studied were positive, which indicates indirect positive selection responses for body weight, fillet yield, and survival can be obtained by selecting for higher body sizes. Such a method can be utilized when it is not possible to obtain body weight and fillet yield records. Using DNA marker based tagging system makes possible to reduce the generation interval and increase the selection intensity, thus improve the selection response. The low average increase in the coefficient of inbreeding per generation indicates that the batch mating method is effective in keeping the inbreeding level low.

References

Acosta, B. O. & Gupta, M. V. (2010). The genetic improvement of farmed tilapias project: Impact and lessons learned. In *Success stories in Asian aquaculture*, pp. 149-171: Springer.

Bentsen, H. B., Eknath, A. E., Palada-de Vera, M. S., Danting, J. C., Bolivar, H. L., Reyes, R. A., Dionisio, E. E., Longalong, F. M., Circa, A. V. & Tayamen, M. M. (1998). Genetic improvement of farmed tilapias: growth performance in a complete diallel cross experiment with eight strains of Oreochromis niloticus. *Aquaculture*, 160 (1): 145-173.

Bentsen, H. B., Gjerde, B., Eknath, A. E., de Vera, M. S. P., Velasco, R. R., Danting, J. C., Dionisio, E. E., Longalong, F. M., Reyes, R. A. & Abella, T. A. (2017). Genetic improvement of farmed tilapias: Response to five generations of selection for increased body weight at harvest in Oreochromis niloticus and the further impact of the project. *Aquaculture*, 468: 206-217.

Bolivar, R. B. & Newkirk, G. F. (2002). Response to within family selection for body weight in Nile tilapia (Oreochromis niloticus) using a single-trait animal model. *Aquaculture*, 204 (3-4): 371-381.

Brzeski, V. J. & Doyle, R. W. (1995). A test of an on-farm selection procedure for tilapia growth in Indonesia. *Aquaculture*, 137 (1): 219-230.

Charo-Karisa, H., Komen, H., Rezk, M. A., Ponzoni, R. W., van Arendonk, J. A. M. & Bovenhuis, H. (2006). Heritability estimates and response to selection for growth of Nile tilapia (Oreochromis niloticus) in low-input earthen ponds. *Aquaculture*, 261 (2): 479-486.

Chiayvareesajja, J., Røed, K. H., Eknath, A. E., Danting, J. C., De Vera, M. P. & Bentsen, H. B. (1999). Genetic variation in lytic activities of blood serum from Nile tilapia and genetic associations with survival and body weight. *Aquaculture*, 175 (1): 49-62.

Clement, S. & Lovell, R. T. (1994). Comparison of Processing Yield and Nutrient Composition of Cultured Nile Tilapia (Oreochromis niloticus) and Channel Catfish (Ictalurus punctatus). *Aquaculture*, 119 (2-3): 299-310.

Coster, A. & Coster, M. A. (2010). Package 'pedigree'. *Book of Abstracts–Oral Presentations–Session*, 3.

de Oliveira, C. A. L., Ribeiro, R. P., Yoshida, G. M., Kunita, N. M., Rizzato, G. S., de Oliveira, S. N., dos Santos, A. I. & Nguyen, N. H. (2016). Correlated changes in body shape after five generations of selection to improve growth rate in a breeding program for Nile tilapia Oreochromis niloticus in Brazil. *Journal of applied genetics*: 1-7.

Eknath, A. E., Tayamen, M. M., Palada-de Vera, M. S., Danting, J. C., Reyes, R. A., Dionisio, E. E., Capili, J. B., Bolivar, H. L., Abella, T. A. & Circa, A. V. (1993). Genetic improvement of farmed tilapias: the growth performance of eight strains of Oreochromis niloticus tested in different farm environments. *Aquaculture*, 111 (1): 171-188.

FAO. (2017). *Global Aquaculture Production 1950-2015*. Available at: http://www.fao.org/fishery/statistics/global-aquaculture-production/en (accessed: 2 May).

Fitzsimmons, K., Martinez-Garcia, R. & Gonzalez-Alanis, P. (2011). Why tilapia is becoming the most important food fish on the planet. *Better Science, Better Fish, Better Life*: 8.

Gilmour, A., Gogel, B., Cullis, B., Welham, S. & Thompson, R. (2015). ASReml user guide release 4.1 structural specification. *Hemel Hempstead: VSN International Ltd*.

Gjerde, B., Mengistu, S. B., Ødegård, J., Johansen, H. & Altamirano, D. S. (2012). Quantitative genetics of body weight, fillet weight and fillet yield in Nile tilapia (Oreochromis niloticus). *Aquaculture*, 342: 117-124.

Gjøen, H. M. (2004). *A new era: The merging of quantitative and molecular genetics—Prospects for tilapia breeding programs*. Proceedings of the sixth international symposium on tilapia in aquaculture.

Haffray, P., Bugeon, J., Rivard, Q., Quittet, B., Puyo, S., Allamelou, J. M., Vandeputte, M. & Dupont-Nivet, M. (2013). Genetic parameters of in-vivo prediction of carcass, head and fillet yields by internal ultrasound and 2D external imagery in large rainbow trout (Oncorhynchus mykiss). *Aquaculture*, 410: 236-244.

Hamzah, A., Ponzoni, R. W., Nguyen, N. H., Khaw, H. L., Yee, H. Y., Nor, M. & Azizah, S. (2014). Performance of the Genetically Improved Farmed Tilapia (GIFT) Strain Over Ten Generations of Selection in Malaysia. *Pertanika Journal of Tropical Agricultural Science*, 37 (4).

Lin, M. (2016). *Statistical model comparison in genetic analysis of challenge test data on Streptococcus agalactiae resistance in Nile tilapia (Oreochromis niloticus)*: Norwegian University of Life Sciences, Ås.

Luan, T. D., Olesen, I., Ødegård, J., Kolstad, K. & Dan, N. C. (2008). *Genotype by environment interaction for harvest body weight and survival of Nile tilapia (Oreochromis niloticus) in brackish and fresh water ponds*. Proceedings from the Eighth International Symposium on Tilapia Aquaculture. 231-240 pp.

Marjanovic, J., Mulder, H. A., Khaw, H. L. & Bijma, P. (2016). Genetic parameters for uniformity of harvest weight and body size traits in the GIFT strain of Nile tilapia. *Genetics Selection Evolution*, 48 (1): 1.

Morrissey, M. B. & Wilson, A. J. (2010). pedantics: an r package for pedigree-based genetic simulation and pedigree manipulation, characterization and viewing. *Molecular Ecology Resources*, 10 (4): 711-719.

Nguyen, N. H., Khaw, H. L., Ponzoni, R. W., Hamzah, A. & Kamaruzzaman, N. (2007). Can sexual dimorphism and body shape be altered in Nile tilapia (Oreochromis niloticus) by genetic means? *Aquaculture*, 272: S38-S46.

Nguyen, N. H., Ponzoni, R. W., Abu-Bakar, K. R., Hamzah, A., Khaw, H. L. & Yee, H. Y. (2010). Correlated response in fillet weight and yield to selection for increased harvest weight in genetically improved farmed tilapia (GIFT strain), Oreochromis niloticus. *Aquaculture*, 305 (1): 1-5.

Ponzoni, R. W., Hamzah, A., Tan, S. & Kamaruzzaman, N. (2005). Genetic parameters and response to selection for live weight in the GIFT strain of Nile Tilapia (Oreochromis niloticus). *Aquaculture*, 247 (1-4): 203-210.

Ponzoni, R. W., Khaw, H. L. & Yee, H. Y. (2010). GIFT: the story since leaving ICLARM (now known as the WorldFish Center): socioeconomic, access and benefit sharing and dissemination aspects.

Pullin, R. & Capili, J. (1988). *Genetic improvement of tilapias: problems and prospects*. The second international symposium on tilapia in aquaculture. ICLARM Conference Proceedings. 259-266 pp.

R Core Team. (2016). *R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing*.

Rezk, M. A., Ponzoni, R. W., Khaw, H. L., Kamel, E., Dawood, T. & John, G. (2009). Selective breeding for increased body weight in a synthetic breed of Egyptian Nile tilapia, Oreochromis niloticus: response to selection and genetic parameters. *Aquaculture*, 293 (3): 187-194.

Rutten, M. J., Bovenhuis, H. & Komen, H. (2005). Genetic parameters for fillet traits and body measurements in Nile tilapia (Oreochromis niloticus L.). *Aquaculture*, 246 (1): 125-132.

Rutten, M. J. M., Bovenhuis, H. & Komen, H. (2004). Modeling fillet traits based on body measurements in three Nile tilapia strains (Oreochromis niloticus L.). *Aquaculture*, 231 (1-4): 113-122.

Rutten, M. J. M., Komen, H. & Bovenhuis, H. (2005). Longitudinal genetic analysis of Nile tilapia (Oreochromis niloticus L.) body weight using a random regression model. *Aquaculture*, 246 (1-4): 101-113.

Skaarud, A., Woolliams, J. A. & Gjøen, H. M. (2014). *A DNA-marker based breeding scheme to utilize the large number of full-sibs in fish species*: Norwegian University of Life Sciences, Ås. Unpublished manuscript.

Thodesen, J., Rye, M., Wang, Y. X., Yang, K. S., Bentsen, H. B. & Gjedrem, T. (2011). Genetic improvement of tilapias in China: Genetic parameters and selection responses in growth of Nile tilapia (Oreochromis niloticus) after six generations of multi-trait selection for growth and fillet yield. *Aquaculture*, 322: 51-64.

Thodesen, J., Rye, M., Wang, Y. X., Bentsen, H. B. & Gjedrem, T. (2012). Genetic improvement of tilapias in China: Genetic parameters and selection responses in fillet traits of Nile tilapia (Oreochromis niloticus) after six generations of multi-trait selection for growth and fillet yield. *Aquaculture*, 366: 67-75.

Thodesen, J., Rye, M., Wang, Y. X., Li, S. J., Bentsen, H. B. & Gjedrem, T. (2013). Genetic improvement of tilapias in China: Genetic parameters and selection responses in growth, pond survival and cold-water tolerance of blue tilapia (Oreochromis aureus) after four generations of multi-trait selection. *Aquaculture*, 396: 32-42.

Trọng, T. Q., Mulder, H. A., van Arendonk, J. A. & Komen, H. (2013). Heritability and genotype by environment interaction estimates for harvest weight, growth rate, and shape of Nile tilapia (Oreochromis niloticus) grown in river cage and VAC in Vietnam. *Aquaculture*, 384: 119-127.

Turra, E. M., de Oliveira, D. A. A., Valente, B. D., Teixeira, E. D., Prado, S. D., de Alvarenga, E. R., de Melo, D. C., Felipe, V. P. S., Fernandes, A. F. A. & Silva, M. D. E. (2012). Longitudinal genetic analyses of fillet traits in Nile tilapia Oreochromis niloticus. *Aquaculture*, 356: 381-390.

Wickham, H. & Francois, R. (2015). dplyr: A grammar of data manipulation. *R package version 0.4*, 1: 20.

Wickham, H. (2016). *ggplot2: elegant graphics for data analysis*: Springer.

Yalew, D. W. (2007). *Genetic and phenotypic parameter estimation in selected farm tilapia population (Oreochromis niloticus)*: Norwegian University of Life Sciences, Ås.

Zak, T., Deshev, R., Benet-Perlberg, A., Naor, A., Magen, I., Shapira, Y., Ponzoni, R. W. & Hulata, G. (2014). Genetic improvement of Israeli blue (Jordan) tilapia, Oreochromis aureus (Steindachner), through selective breeding for harvest weight. *Aquaculture Research*, 45 (3): 546-557.

Supplementary materials

Supplementary material 1. The standardization of the phenotypes for body weight in R

```
gst <- read.csv("gst.fs.fy.csv", header=T)   # reading the data

gst$Environment <- paste(gst$Generation, gst$Batch, sep="_")     #Giving a distinct name to each batch. This was required as batches were numbered similarly inside their generations.


library(dplyr)    # Data manipulations were performed using the “dplyr” package


gst <- gst %>% arrange(Environment, Weight)  # The fish was ordered by their environment and weight


weight.summary <- gst %>% group_by(Environment) %>% summarise(mn=mean(Weight, na.rm=T), stdev=sd(Weight, na.rm=T))  # Calculation of the standard deviations of each batch


gst$sd.weight<-
weight.summary$stdev[match(gst$Environment,weight.summary$Environment)]
#Importing the standard deviation of batches into the original data


gst$Weight_C <- gst$Weight / gst$sd.weight  #Standardized phenotypes


### The codes for other traits are the same, thus they are not given.
```

Supplementary material 2. Variance components and heritability calculation for body weight in ASReml

```
Corrected Weight All Generations
Animal !A !P
Sire !A !P
Dam !A !P
Sex !A !L M F
Generation !I
Environment !A
Weight
Weight_C

pedigree.csv !SKIP 1 !ALPHA !MAKE

Weight_C.csv !SKIP 1

Weight_C ~ mu Sex Environment !r Animal fac(Sire,Dam)

Residual units

VPREDICT !DEFINE
F VarA Animal
F VarC fac(Sire,Dam)
F VarP VarA+VarC+Residual
H h2 VarA VarP
H c2 VarC VarP
```

Supplementary material 3. Variance components and heritability calculation for fillet yield in ASReml

```
Fillet Yield Clean Data
Animal !A !P
Sire !A !P
Dam !A !P
Sex !A !L M F
Generation !I
Environment !A
Weight
Weight_C
Fillet_Yield
Filleter !A

pedigree.csv !SKIP 1 !ALPHA !MAKE

FY_Clean.csv !SKIP 1 !MVINCLUDE !DDF

Fillet_Yield ~ mu Sex Environment Filleter !r Animal fac(Sire,Dam)

Residual units

VPREDICT !DEFINE
F VarA Animal
F VarC fac(Sire,Dam)
F VarP VarA+VarC+Residual
H h2 VarA VarP
H c2 VarC VarP
```

Supplementary material 4. Variance components and heritability calculation for survival in ASReml

Survival Model

```
Environment !A
Generation !I
Survival
Sire !A !P
Dam !A !P
Num_Obs !I
Fam_No !A
Num_Ent !I

pedigree.csv !SKIP 1 !ALPHA

survival_family.csv !SKIP 1 !MAXIT 900 !CONTINUE !DDF

Survival !BIN !PROBIT !TOTAL=Num_Ent ~ mu Environment !r Sire and(Dam,1)

Residual units
VPREDICT !DEFINE
F VarA Sire*4
F VarP VarA+Residual
H h2 VarA VarP
```

Supplementary material 5. Removing the Outliers

```
outliers<-read.csv("outliers.csv",header=T)  #Outliers are imported from ASReml
fy<-read.csv(“fy.csv”,header=T)
fy.clean<-fy[-outliers$order,]
write.csv(fy.clean,"FY_Clean.csv")
```

Supplementary material 6. Calculating the genetic correlations

```
ebv <- read.csv("ebvs.csv", header=T)
w.fy <- cor.test(ebv$EBV.W, ebv$EBV.FY)
w.fw <- cor.test(ebv$EBV.W, ebv$EBV.FW)
w.le <- cor.test(ebv$EBV.W, ebv$EBV.LE)
w.de <- cor.test(ebv$EBV.W, ebv$EBV.DE)
w.th <- cor.test(ebv$EBV.W, ebv$EBV.TH)
w.s <- cor.test(ebv$EBV.W, ebv$EBV.Survival)
#The codes are similar for other traits…


cor.test.plus <- function(x) {
  list(x,
       Standard.Error = unname(sqrt((1 - x$estimate^2)/x$parameter)))
}
cor.test.plus(w.fy)  # For calculation of standard errors  #The code is  similar for other traits…
```

Supplementary material 7. Calculating the phenotypic correlations

```
full.data <- read.csv("gst.csv", header=T)  #Data file with standardized phenotypes
data.2 <- full.data[,c(14,8,15:18)]
w.fy <- cor.test(data.2$Weight_C, data.2$Fillet.Yield)
w.fw <- cor.test(data.2$Weight_C, data.2$FW_C)
w.le <- cor.test(data.2$Weight_C, data.2$Length_C)
w.de <- cor.test(data.2$Weight_C, data.2$Depth_C)
w.th <- cor.test(data.2$Weight_C, data.2$Thickness_C)
fy.fw <- cor.test(data.2$Fillet.Yield, data.2$FW_C)
fy.le <- cor.test(data.2$Fillet.Yield, data.2$Length_C)
fy.de <- cor.test(data.2$Fillet.Yield, data.2$Depth_C)
fy.th <- cor.test(data.2$Fillet.Yield, data.2$Thickness_C)
fw.le <- cor.test(data.2$FW_C, data.2$Length_C)
fw.de <- cor.test(data.2$FW_C, data.2$Depth_C)
fw.th <- cor.test(data.2$FW_C, data.2$Thickness_C)
le.de <- cor.test(data.2$Length_C, data.2$Depth_C)
le.th <- cor.test(data.2$Length_C, data.2$Thickness_C)
de.th <- cor.test(data.2$Depth_C, data.2$Thickness_C)



cor.test.plus <- function(x) {
  list(x,
       Standard.Error = unname(sqrt((1 - x$estimate^2)/x$parameter)))
}
cor.test.plus(w.fy)  # For calculation of standard errors  #The code is  similar for other traits…
```

Supplementary material 8. Calculating the inbreeding coefficients

```
library(pedigree)
ped <- read.csv("pedigree.csv", header=T)
F<- calcInbreeding(ped)
ped$F<-F
Ave.Inb<-ped%>%group_by(Gen)%>%summarise(Av.In=mean(F))
```

Supplementary material 9. The descriptive statistics for each trait after the outlier phenotypes are removed

Table S-1. The number of individuals (n) in each generation, means and coefficient of variations (CV %) of trait body weight after the outliers are deleted

Generation	n	Mean Weight	CV Weight
14	3694	301.0	44.7
15	2316	252.2	33.2
16	1799	247.0	29.9
17	3246	631.3	25.5
18	1218	528.7	30.4
19	4102	689.4	25.9
20	3471	680.2	26.5
21	4238	715.3	21.3
22	5010	790.7	31.8
23	2284	766.7	37.6
24	2532	729.8	37.8
Overall	33910	607.9	44.6

Table S-2. The number of individuals (n) in each generation, means and coefficient of variations (CV %) of trait fillet yield after the outliers are deleted

Generation	n	Mean FY	CV FY
17	3187	38.3	6.2
18	3481	38.1	5.8
19	4080	38.0	5.8
20	3433	37.9	6.2
21	4206	37.6	6.2
22	5002	37.8	5.5
23	2262	37.4	6.6
24	2522	37.1	6.9
25	3789	37.2	5.9
Overall	31962	37.7	6.1

Table S-3. The number of individuals (n) in each generation, means and coefficient of variations (CV %) of trait fillet weight after the outliers are deleted

Generation	n	Mean FW	CV FW
17	3248	243.0	28.2
18	3735	220.4	27.3
19	4109	262.4	28.8
20	3478	257.8	29.3
21	4244	268.7	24.7
22	5027	298.6	34.4
23	2287	287.6	40.2
24	2533	271.3	40.7
25	3806	270.9	37.8
Overall	32467	265.3	33.9

Table S-4. The number of individuals (n) in each generation, means and coefficient of variations (CV %) of trait length after the outliers are deleted

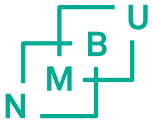
Generation	n	Mean Length	CV Length
14	338	25.03	7.23
15	2115	17.93	9.96
16	1800	17.97	7.81
17	3227	24.12	7.49
18	3505	23.09	8.25
19	4076	24.69	7.35
20	3444	24.69	8.66
21	4207	25.25	7.64
22	4988	26.06	9.98
23	2274	25.59	12.12
24	2526	25.53	11.20
25	3765	25.98	9.26
Overall	36265	24.3	13.3

Table S-5. The number of individuals (n) in each generation, means and coefficient of variations (CV %) of trait depth after the outliers are deleted

Generation	n	Mean Depth	CV Depth
14	336	11.2	10.3
15	2116	8.0	13.6
16	1800	7.8	11.0
17	3239	11.1	9.3
18	3516	10.6	9.9
19	4093	11.3	9.2
20	3465	11.2	10.4
21	4219	11.4	8.4
22	4997	11.7	11.4
23	2275	11.6	14.4
24	2526	11.2	15.5
25	3779	11.5	12.2
Overall	36361	10.9	15.1

Table S-6. The number of individuals (n) in each generation, means and coefficient of variations (CV %) of trait thickness after the outliers are deleted

Generation	n	Mean Thickness	CV Thickness
14	339	5.4	10.5
15	516	4.3	8.9
17	3239	35.4	39.2
18	3528	40.9	9.3
19	4088	43.9	7.4
20	3448	44.3	7.9
21	4213	46.0	5.9
22	4989	48.4	11.5
23	2215	52.5	12.7
24	2526	49.9	15.1
25	3782	48.8	9.2
Overall	32883	44.3	22.6



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