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Coalescing and discriminating offspring from pure line and reciprocal crosses of two African catfishes: *Clarias gariepinus* and *Heterobranchus longifilis*

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ABSTRACT

The current research involved pure and reciprocal crosses of two African catfishes: *Clarias gariepinus* (Cl) and *Heterobranchus longifilis* (Ht) to give four groups namely Cl×Cl, Cl×Ht, Ht×Cl and Ht×Ht. Triplicate groups of progenies were reared for 56 days and length (total length) were taken weekly. Mortalities were recorded and growth shooters sorted. Data was corrected for missing values (mortality) using imputation algorithm. Data passed the Kaiser-Meyer-Olkin factor adequacy test (Measure of Sampling Adequacy (MSA)>0.8) and the Bartlett's test of sphericity ($p<0.05$) prior to principal component analysis, linear discriminant analysis and hierarchical clustering. For all samples, length from weeks 2 and 3 was 42.2% of variation –(dimension 1) which we call *archikotic* growth phase and from weeks 7 and 8 accounted for 11.1% (dimension 2) which we call *telikotic* growth phase are grouping factors. For non-growth shooters, length from weeks 1 to 5 (83.5% - dimension 1) named *endiametic* growth phase and from week 7 - 8 (6.3% - dimension 2) named as *telikotic* growth phase are the grouping factors. Growth shooters can be grouped based on length between week 2 and 6 (75% variation in dimension 1 - *endiametic* growth phase) and week 1 and 8 (10.2% variation in dimension 2 - *architelic* growth phase). Accuracy in the identification of progeny according to their family declined with uniformity in size. Grouping of the crosses also showed that there was a maternal influence on the clustering such that Cl×Cl and Cl×Ht clustered together as did Ht×Ht and Ht×Cl.

INTRODUCTION

The mating of individuals that can be well differentiated in terms of their genetic makeup is called hybridization **(Bartley *et al*., 2000)**, hence inter-generic hybridization **(Ataguba *et al*., 2010; Ndimele *et al*., 2011; Okomoda *et al*., 2018)**. Crossbreeding refers to pairing of animals that are actually different breeds and breeds refers to lineages within a species that exhibit variation in gene frequencies **(Bondoc, 2008)**, It is common to see the use of hybridization in tandem with crossbreeding in the fields of agriculture and aquaculture with the ultimate **and Gorda, 1995)**, tilapia **(De Verdal *et al*., 2014)** and the African catfish **(Adene *et al*., 2017; Oben *et al*., 2015)**.

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The desired goal in each crossbreeding and hybridization trials is heterosis, a term that extends beyond hybrid vigor into increased genetic variability, fast growth, and greater biomass production **(Bondoc, 2008)**. aim of improvement in quantitative (growth, disease resistance etc.) or qualitative (structure, form etc.), traits with examples in the channel catfish **(Smitherman *et al*., 1996)**, common carp **(Bakos**

One rationale behind this hypothesis is that different species are likely to have evolved different alleles at common gene loci and thus that there will predictably be high levels of heterozygosity in the hybrid progeny **(Zhang *et al*., 2015)**. High levels of heterozygosity are often associated with greater fitness **(Danzmann *et al*., 1988)**. Consistency in the desired trait in the F_1 generation is an indication that

hybrid vigor was established and is the basis for providing predictable gains in the hybrids. The other main rationale for carrying out hybridization is to combine a set of desirable characteristics from two or more different species into a single hybrid with the combination of traits in the hybrid then having significant benefits **(Bondoc, 2008)**, in the context of production or marketing, over either of the parental species. Hybridization is widely used to increase growth rate **(De Verdal *et al*., 2014)**, survival rate **(Ataguba *et al*., 2010)**, manipulate sex ratios **(Desprez *et al*., 2006)**, produce sterile animals **(Yoshikawa *et al*., 2018)**, improve flesh quality **(Jankowska *et al*., 2007)**, increase disease resistance **(Wolters *et al*., 1996)**, and improve environmental tolerance **(Kelly and Kohler, 1999)**.

Hybridization and crossbreeding are two relatively simple techniques that terminate at the first filial (F_1) generation, but subsequent display of inherited vigor is premised upon selection. Selection uses input from crossbreeding or hybridization to obtain germplasm. Here, there is no new gene pool generated rather the parental lines selected are procreated to have more progeny and increase allelic frequency for desired traits **(Bondoc, 2008)**. It is clear to see that selection involves the use of genotypes with expressed phenotypes that are superior in breeding so that subsequent generations will carry the superior genes in numbers that are more than the parental lines.

The principles of selection are easy to adopt in terrestrial animal agriculture and in the aquaculture of some species such as tilapia and carp where male and female line selection is easy since milt is easily obtained, or natural spawning takes place. In the African catfish, the sire line is usually eliminated in the process of breeding hence when heterosis is achieved in the F_1 generation, the backcrossing of the offspring with the parental sire is impossible except if milt is cryo-preserved. Backcrossing is ideal for knocking out some genes since 50% of the genes will be affected by heterosis **(Benavides and Guénet, 2012)**, hence traits like cannibalism and aggression can be reduced by half with a generation of offspring of uniform size **(Marjanovic *et al*., 2016)**.

Selection therefore becomes the key tool for genetic improvement in both pure line and reciprocal hybrid African catfish. Selection protocols can involve individual selection, pedigree selection, progeny testing and sib-information **(Bondoc, 2008)**. These methods require information on desired traits. In selecting for growth rate that leads to uniformity in size, it is important to have information on the temporal progression of growth of the F_1 generation to identify size uniformity performers and if the goal is fast growth, growth shooters can easily be identified.

Multivariate analysis comprises a suite of statistical tools that are used to explain results of experiments involving multiple measurements on each experimental unit such that the relationship between the multivariate metrics and their structure fully explain the results of the study **(Olkin and Sampson, 2001)**. Multivariate statistical techniques include factor analysis which encompasses principal component analysis, correspondence analysis, and multiple correspondence analysis as well as cluster analysis. Principal component analysis (PCA) groups *variables* **(Kassambara, 2017b)**, while cluster analysis groups *observations* **(Kassambara, 2017a)**. The distinction between these two methods is important because it gives backing to the goal of any research. Principal component analysis is a supervised grouping algorithm that considers the variables and leaves grouping as it were in its input while cluster analysis is unsupervised grouping algorithm that seeks to group observations without recourse to the input groups. Clustering is a powerful tool that identifies latent relationships in data before grouping them. Clustering finds application in biological science and biotechnology with attendant use in phylogeny, molecular genetics, proteomics, and clinical diagnosis **(Zhao and Karypis, 2003)**.

The current research utilized the multivariate analysis tools of PCA and cluster analysis to show the categories and factors (temporal progression in length) that contribute to the categorization of the progeny from the crosses. This is a classical attempt at delineating progeny for further selection into a breeding program.

MATERIALS AND METHODS

2.1. Broodstock and breeding

Broodstock (6 ♀*C. gariepinus*, 6 ♀*H. longifilis*, 6 ♂*C. gariepinus* and 6 ♂*H. longifilis*) were obtained from the

University of Agriculture Makurdi, Department of Fisheries and Aquaculture Teaching and Research Farm and kept separately in indoor concrete tanks designated for males and females. The weights of the broodstock were recorded Table 1. Mating combination was carried out to yield, triplicate for each cross in the following order (♀× ♂):

- *C. gariepinus* × *C. gariepinus* (Cl × Cl)
- *H. longifilis* × *H. longifilis* (Ht × Ht)
- *H. longifilis* × *C. gariepinus* (Ht × Cl)
- *C. gariepinus* × *H. longifilis* (Cl × Ht)

Table 1. Broodstock weight and volume of hormone (Ovaprim) administered

S/No.	Females				Males (g)	
	Cl (g)	VH (ml)	Ht (g)	VH (ml)	Cl	Ht
1	650	0.33	600	0.30	600	530
2	620	0.31	640	0.32	550	525
3	570	0.29	620	0.31	520	590
4	630	0.32	650	0.33	520	530
5	550	0.28	580	0.29	560	580
6	650	0.33	560	0.28	580	550

VH= Volume of Ovaprim administered

Final oocyte maturation and ovulation was achieved via the administration of a single intramuscular injection of Ovaprim (0.5 ml kg^{-1}). After the administration of the hormone, male and female fish were returned to their respective holding tanks to prevent natural spawning and male aggressiveness. Average latency period was 12 hours and 15 hours for *C. gariepinus* and *H. longifilis* respectively. After expiration of the latency period, oocytes were collected by applying a gentle forward push on the abdomen in the direction of the caudal fin into a dry clean plastic bowl. Milt was obtained via surgical removal of the testes. The testes were cut open using a sharp surgical blade. Milt was extended in 0.9% NaCl solution. Ova was fertilized using the wet fertilization method. The extended milt mixture was quickly added to the eggs and stirred using a feather. Saline solution (0.9% NaCl) was added to the resulting mixture of egg and extended milt. The resulting mixture was stirred using a feather for one minute after which fertilized eggs were incubated in triplicate batches inside 12 plastic aquaria of 60L capacity /each.

Upon hatching, fry was removed from the incubation substrate and the dead eggs washed off before they were again returned into the plastic tanks this time in batches of 500 randomly selected fry per tank as assigned to each cross. These were nursed for two weeks and fed on decapsulated Artemis for the start before being weaned to starter feed at day 10. At the end of two weeks, the surviving fry were again harvested totally from the tanks and then restocked in batches of 100 randomly selected fry per tank to set the stage for measurement of length.

The total lengths of the progenies were taken every week using a millimeter ruler. The fish were fed *ad libitum* twice daily except on sampling days. Larger fish were sorted out as growth shooters using the criteria of **(Ataguba *et al*., 2022)** where an individual is removed if its total length became approximately greater than the sum of the current weeks mean length and difference between the current weeks mean length and the preceding week's mean length. This can be represented as:

$$C_T = \mu_2 + (\mu_2 - \mu_1)$$

Where:

CT Shooter cutoff length

μ1 Previous week's mean length

μ2 Current Week's mean length

2.2. Water Quality

Water quality in the holding tanks was monitored for dissolved oxygen (DO), temperature, alkalinity, biochemical Oxygen Demand (BOD) and pH. Water quality parameters such as pH and Dissolved Oxygen were monitored using Hanna Multiparameter Water Quality Probe (Model HI-98129). A mercury in glass thermometer was used to take temperature readings. The 5-day dark bottle test was used to determine biochemical Oxygen Demand (BOD) **(APHA, 2005)**.

Alkalinity was determined using the phenolphthalein titration method **(APHA, 2005)**.

2.3. Data analysis

Descriptive statistics of spread of length was determined using Minitab 14®. Length data were processed for missing values as a result of mortality using the powerful imputation algorithm of mice package **(Van Buuren and Groothuis-Oudshoorn, 2011)** in R v. 3.4.3 **(R Core Team, 2017)**. Principal component analysis to group the temporal length values that explain the variation in the size of the progeny was done using factoextra package in R **(Kassambara and Mundt, 2017)** in tandem with the package cluster **(Maechler *et al*., 2017)** which was also used to group the crosses and find their relationship as determined by temporal growth in length. Before carrying out PCA, data was checked for sample adequacy using the Kaiser-Meyer-Olkin (KMO) Test as well as for sphericity using the Bartlett's sphericity test which tests if correlations between variables is greater than what is expected by chance. These tests were run using the psych package in R **(Revelle, 2017)**. The Kaiser-Meyer-Olkin factor adequacy values were >0.8 which was well above the 0.5 threshold specified by **Zillmer and Vuz (2013)** while the Bartlett's test of sphericity was all significant ($p<0.05$) which effectively nullifies the null hypothesis that all off-diagonal correlations are zero **(Nakazawa, 2011)** and therefore data can be analyzed using PCA. Linear Discriminant Analysis (LDA) was later performed on the data using Minitab 14® to check the accuracy of classification of progeny into the various crosses based on their length data as well as the classification of fish as growth shooters and non-growth shooters.

RESULTS

3.1 Descriptive

The spread of total length of the all progenies in each cross Table 2 shows that initial maximum length of the cross Ht×Cl was the highest (37mm) while the least initial maximum length (18mm) was recorded for the pure line cross of Ht×Cl. Final total length showed that the crosses Cl×Cl and Cl×Ht had the highest maximum length (48mm) while Ht×Cl and Ht×Ht had similar final maximum lengths of 45.5mm and 45mm respectively. The total mortality number was highest in the cross Ht×Cl (140 fish) and lowest in Cl×Cl (78 fish).

Table 2. Spread of length among all progeny

Variable	Cross	N	N*	Minimum	Median	Maximum
Initial Length (mm)	Cl × Cl	300	0	10.00	17.00	28.00
	Cl × Ht	300	0	11.00	19.00	29.00
	Ht × Cl	300	0	9.00	13.00	37.00
	Ht × Ht	300	0	8.00	12.00	18.00
Final Length (mm)	Cl × Cl	63	78	33.00	40.00	48.00
	Cl × Ht	43	109	26.00	40.00	48.00
	Ht × Cl	48	140	26.00	39.50	45.50
	Ht × Ht	58	124	25.50	33.25	45.00

*= Final Number of Mortalities

When the growth shooters are excluded from the lot Table 3, the maximum total length of progenies of the cross Cl×Cl and that of Ht×Cl as at the first week of sampling were equal (30mm) while the cross Ht×Ht had the least maximum total length (19mm). Maximum values for final total length among progenies that were devoid of growth shooters was equal (43mm) for three crosses: Cl×Cl, Cl×Ht and Ht×Ht while Ht×Ht had maximum total length of 42mm.

Table 3. Spread of length among uniform sized fish

Variable	Cross	N	Minimum	Median	Maximum
Length /Week 1 (mm)	Cl×Cl	265	11.500	23.000	30.000
	Cl×Ht	214	14.000	20.000	28.000
	Ht×Cl	201	11.000	16.000	30.000
	Ht×Ht	182	11.000	15.000	19.000
Length/ Week 8 (mm)	Cl×Cl	51	33.000	39.000	43.000
	Cl×Ht	34	26.000	39.000	43.000
	Ht×Cl	41	26.000	39.000	43.000
	Ht×Ht	49	25.500	32.000	42.000

At the beginning of sorting of growth shooters Table 4, maximum total length was 52mm in the cross Ht×Cl and least in the cross Ht×Ht (23mm). The number of growth shooters was however more among progeny of the cross Cl×Ht (21 fish). The total number of growth shooters at the end of the experiment was highest in the cross Cl×Cl (159 fish) and least in the cross Ht×Cl (112 fish). Maximum total length at the end of the trials was lower than at inception and is effectively the same as the values for all progeny combined Table 2. The median values of length among growth shooters however are greater than the values for all progeny combined. Final median value for total length among the growth shooters was least in progeny from the cross Ht×Ht

(37.5mm) and highest for progeny from the cross Cl×Ht (45mm).

Table 4. Spread of length among growth shooting fish

Variable	Cross	Cumulative N	Minimum	Median	Maximum
Length Week 1 (mm)	Cl×Cl	18	29.00	32.00	38.50
	Cl×Ht	21	22.00	31.00	42.00
	Ht×Cl	20	18.00	24.50	52.00
	Ht×Ht	14	20.00	21.00	23.00
Length Week 8 (mm)	Cl×Cl	159	39.50	44.00	48.00
	Cl×Ht	148	40.00	45.00	48.00
	Ht×Cl	112	38.00	44.00	45.50
	Ht×Ht	118	36.00	37.50	45.00

3.2 Multivariate classification

The total lengths from initial measurement to week 6 are separated effectively as one factor that can be used to differentiate the progenies of the crosses Fig. 1 while total lengths for week 7 and week 8 serve as the second discriminant factor. Within the first discriminant factor (Dim 1), total length in week 2 (15.13%) and week 3 (13.57%) contributed more to the factor loading Fig. 1, Table 5 while the total lengths for week 7 (27.31%) and 8 (51.64%) contributed heavily to the second discriminant factor (Dim 2).

Table 5. Contribution (%) of variables to first 2 dimensions (Dim) by all samples

Variables	Dim.1	Dim.2
Initial	11.02	0.14
Week.1	10.88	8.34
Week.2	15.13	3.57
Week.3	13.57	3.95
Week.4	13.12	0.00
Week.5	13.00	0.02
Week.6	10.17	5.03
Week.7	8.49	27.31
Week.8	4.60	51.64

Among the progenies that were not growth shooters Fig. 2, lengths for week 1 through week 5 contributed heavily to the first discriminant factor (Dim 1) which accounts for 83.5% of the variation in the total lengths of non-growth shooters observed throughout the study. Dimension 2 is determined mainly by total length at week 7 and 8 as well with 22.27% and 40.86% contributions respectively Table 6.

Table 7. Dimension contributions (%) growth shooters

Variables	Dim.1	Dim.2
Week.1	8.67	46.70
Week.2	14.02	0.35
Week.3	14.16	10.04
Week.4	13.13	15.76
Week.5	15.14	0.78
Week.6	13.51	2.87
Week.7	11.75	0.16
Week.8	9.63	23.35

The variables that define the growth shooting phenomenon as coalesced into 2 dimensions Fig. 4 indicates that dimension 1 is characterized by total length between week 2 and week 5 Table 8 while dimension 2 is explained heavily by total length in week 7 (23.03%) and week 8 (44.59%).

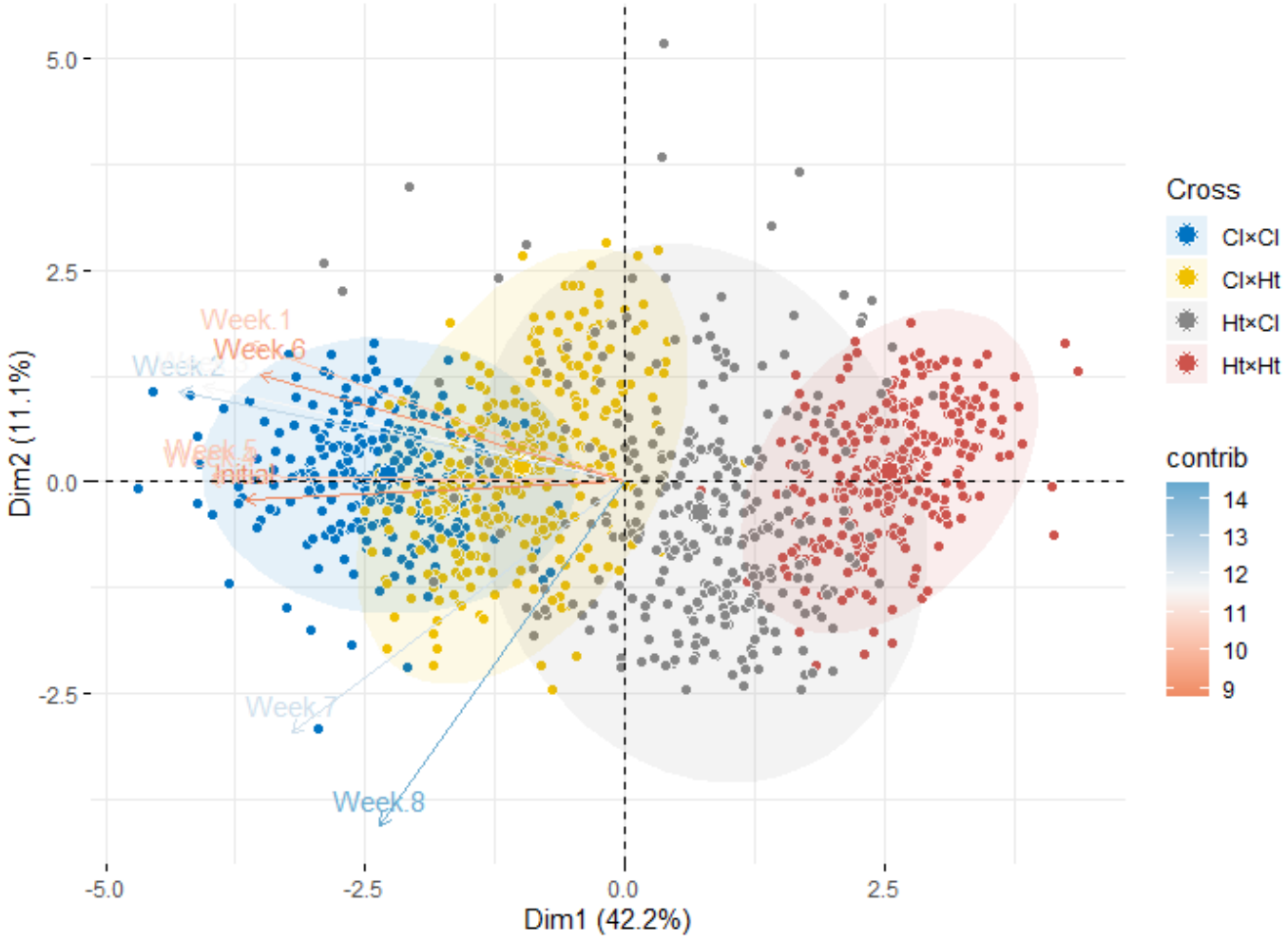

Fig. 2. PCA biplot for non-growth shooters

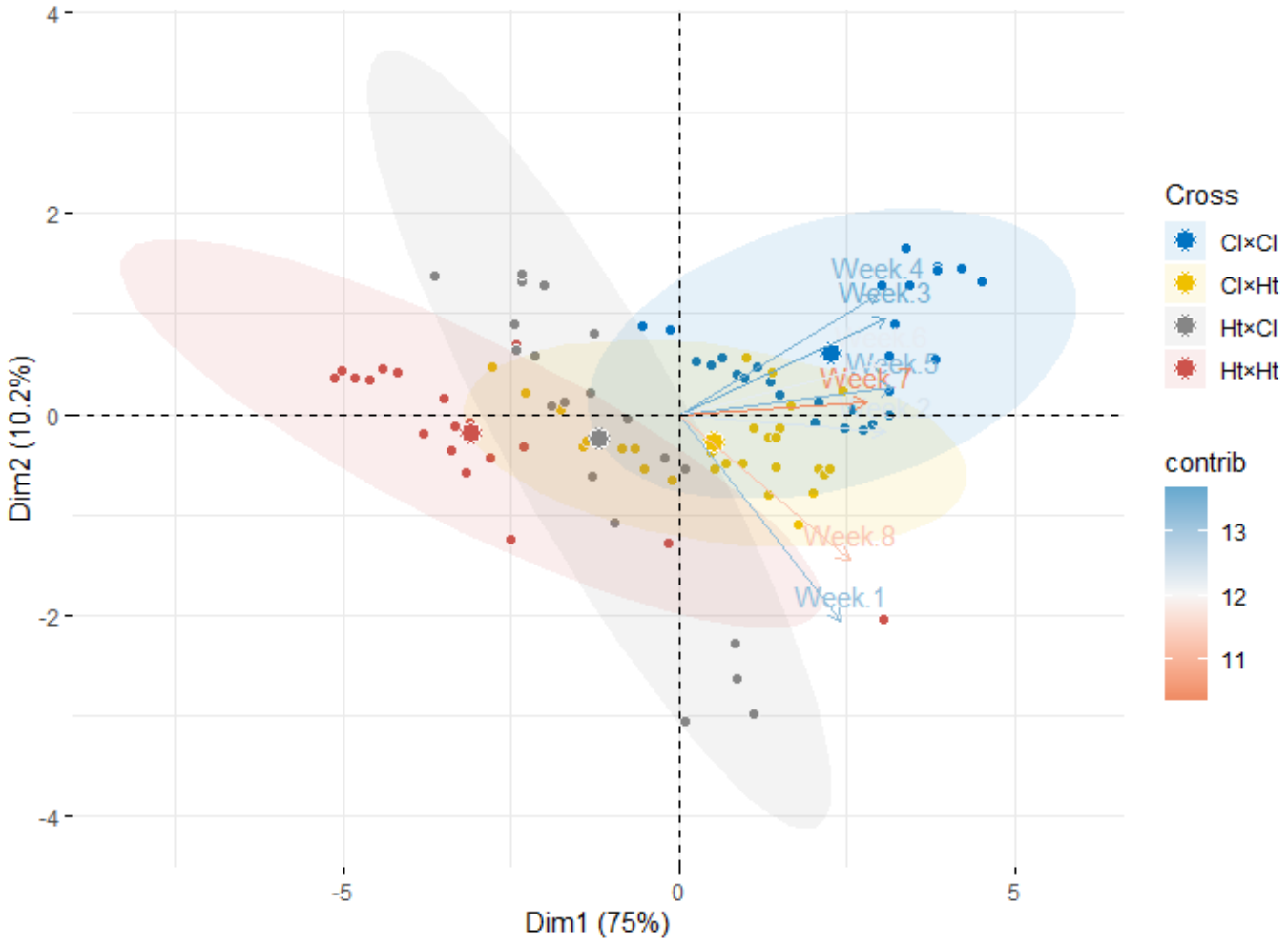

Fig. 3. PCA biplot for growth shooters from all crosses

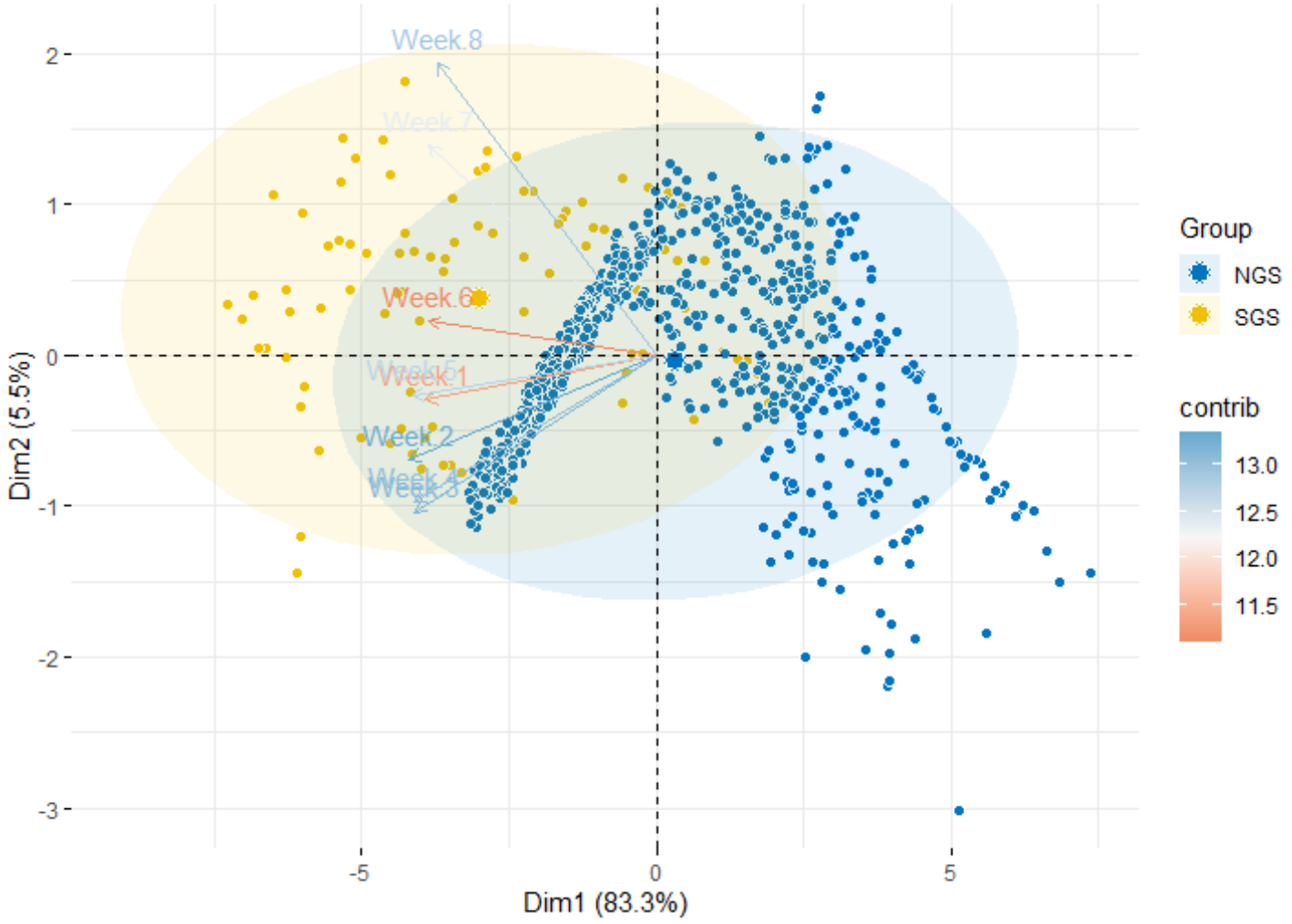

Fig. 4. PCA biplot sowing loadings for growth shooting regardless of crosses (NGS = Non-Growth shooters; SGS = Sorted Growth Shooters).

Table 8. Dimension contributions (%) for discerning growth shooters

Variables	Dim.1	Dim.2
Week.1	12.00	1.05
Week.2	13.85	5.71
Week.3	13.18	12.93
Week.4	13.18	11.24
Week.5	13.49	0.85
Week.6	11.78	0.60
Week.7	11.67	23.03
Week.8	10.86	44.59

3.3 Discriminant analysis

Linear discriminant analysis (LDA) of all progenies regardless of growth shooting status Table 9 reveals 86% success in classification for Cl×Cl, 85% for Cl×Ht, 75% for Ht×Cl and 94% for Ht×Ht. Overall there was 85% accuracy in grouping the progeny.

Table 9. Discriminant grouping of progenies of reciprocal crosses of *C. gariepinus* and *H. longifilis*

	True Group			
Put into Group	**Cl×Cl**	**Cl×Ht**	**Ht×Cl**	**Ht×Ht**
Cl×Cl	257	34	1	0
Cl×Ht	43	255	47	0
Ht×Cl	0	11	225	18
Ht×Ht	0	0	27	282
Total N	300	300	300	300
N correct	257	255	225	282
Proportion	0.86	0.85	0.75	0.94
N	**Correct**		**Proportion**	
1200	1019		0.85	

When growth shooters are removed from the population, accuracy at discriminating between progenies of the crosses reduced Table 10. The accuracy of prediction for Cl×Cl declined to 655 while that for the hybrid Cl×Ht declined even more to settle at 38%. Detection accuracy for Ht×Cl fell to 58% and that of Ht×Ht reduced to 75%. The overall accurscy of delineating non-growth shooters in each cross was 59%

Table 10. Discriminant grouping of non-growth shooting progeny of reciprocal crosses of *C. gariepinus* and *H. longifilis*

	True Group			
Put into Group	**Cl×Cl**	**Cl×Ht**	**Ht×Cl**	**Ht×Ht**
Cl×Cl	172	99	20	0
Cl×Ht	61	81	27	10
Ht×Cl	23	5	117	35
Ht×Ht	9	29	37	137
Total N	265	214	201	182
N correct	172	81	117	137
Proportion	0.65	0.38	0.58	0.75
N	**Correct**		**Proportion**	
862	507		0.59	

Accuracy at predicting the origin of progeny as regards the cross, was perfect for growth shooters of the cross Cl×Cl (100%) with the maternal *C. gariepinus* hybrid cross Cl×Ht having an accuracy of 86% while accuracy for Ht×Cl was 75% and that for Ht×Ht was 89%. Overall accuracy at predicting shooters in each cross was 88% Table 11.

Table 11. Discriminant grouping of growth shooting progeny of reciprocal crosses of *C. gariepinus* and *H. longifilis*

	True Group			
Put into Group	**Cl×Cl**	**Cl×Ht**	**Ht×Cl**	**Ht×Ht**
Cl×Cl	28	2	0	0
Cl×Ht	0	25	4	2
Ht×Cl	0	0	15	0
Ht×Ht	0	2	1	16
Total N	28	29	20	18
N correct	28	25	15	16
Proportion	1.00	0.86	0.75	0.89
N	**Correct**		**Proportion**	
95	84		0.88	

Discriminant analysis for shooters revealed that accurate placement of non-growth shooters irrespective of cross was 98% as against 74% for placement of growth shooters Table 12. The accuracy of placement between both groups was 96%.

Table 12. Discriminant grouping of growth shooting progeny of reciprocal crosses of *C. gariepinus* and *H. longifilis*

	True Group	
Put into Group	**NGS**	**SGS**
on-Growth Shooters (NGS)	848	25
Sorted Growth Shooters (SGS)	14	70
Total N	862	95
N correct	848	70
Proportion	0.98	0.74
N	Correct	Proportion
957	918	0.96

The dendrogram of phylogeny of crosses between *C. gariepinus* and *H. longifilis* Fig. 5 shows that the crosses clustered together along the lines of maternal descent with Ht×Ht and Ht×Cl forming a superior clade to Cl×Cl and Cl×Ht.

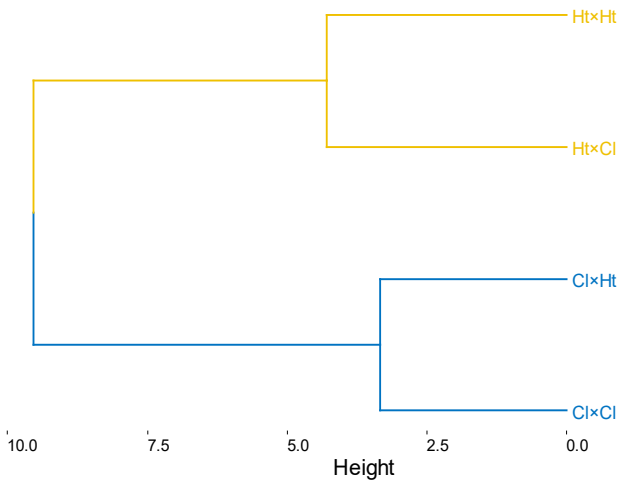

Fig. 5. Dendrogram depicting the joining of clades of crosses between *C. gariepinus* and *H. longifilis*

The water quality Table 13 was quite within the tolerable and recommended ranges. The dissolved oxygen levels were good for tropical freshwater fish with values ≥5.0 mg.l^{-1} (≥80% saturation) **(Mallya, 2007)** while pH which ranged between 7.98 and 8.12 was within the recommended range of 6.0 – 9.0 **(Riffel *et al.*, 2012)**. Mean total alkalinity was >20mg.l^{-1} for all the crosses and were as recommended by **Wurts (2002)**. Biological oxygen demand (BOD) is below the 5mg.l^{-1} threshold as recommended by **Das (1997)** while temperature was within the recommended range of 25-32°C (**Das, 1997**).

Table 13. Water quality parameters during the growth trials involving progeny of reciprocal crosses between *C. gariepinus* and *H. longifilis*.

DISCUSSION

The initial advantage of progeny of the cross Ht×Cl in terms of maximum length indicates the early growth potential of the hybrid within the culture facility **(Solomon and Taruwa, 2011)**. The hybrid Ht×Cl has been named in several literature as Heteroclarias, a name derived from the maternal cum paternal combination of *Heterobranchus* spp and *C. gariepinus* **(Anyanwu *et al.*, 2007; Mayor *et al.*, 2009)**. The final picture of lengths across the genetic groups however went in favour of the lines involving *C. gariepinus*. The mean growth in weight of *C. gariepinus* in groups without sorting has been shown to exceed that of groups with sorting of growth shooters **(Biu *et al.*, 2015)**. This clearly shows that *C. gariepinus* follows classical asymptotic growth pattern with a lag phase at initial step and exponential phase in between before reaching an asymptote **(Mello *et al.*, 2015)**. The trend of length among non-growth shooters at the start of sorting indicates the lack of uniformity in size at the start of the sorting process. However, with sorting, the final lengths of fish across all genetic groups coalesced within 1mm difference indicating the efficiency of the sorting protocol. A similar trend of non-uniformity in size at beginning of sorting was also observed among growth shooters that were identified. At the end of data collection final length of growth shooters fell within 3mm difference from the initial 29mm range.

In the determination of categories of all progenies as well as non-growth shooters based on temporal measurements of length, dimension 1 can correctly be given the nomenclature of *archikotic* (from the Greek word archikós meaning initial) growth phase since the contributions to the dimension comes from length measurements between weeks 2 and 5 while dimension 2 can be called *telikotic* (from the Greek word telikós meaning final) growth phase (contributions from weeks 7 to 8). This trend is consistent with fast growth at fry stage and subsequent slow growth at juvenile stage and it is usually accompanied by reduction in condition factor

(Fonseca and Cabral, 2007). Dimensions for predicting classes of growth shooters from the crosses under study indicates that the first dimension can be named *endiametic* (from the Greek word endiámesos meaning intermediate) growth phase while the second dimension can be named '*architelic*' phase drawing from the Greek words for beginning (archí) and end (télos) since it is derived mainly from lengths in weeks 1 and 8. When all progenies are coalesced, there is the presence of outliers within the ellipses signifying the data points for growth shooters as well as growth laggers. The uniformity and inclusive nature of all data points in the ellipses for non-growth shooters signifies the high accuracy of agglomeration of the offspring based on temporal changes in length. Growth shooters were well classified according to the loading of length over the study period. Size difference can be an advantage in the wild **(Costa-Pereira *et al*., 2018)** but is the foundation of cannibalism in aquaculture **(Baras and Fortuné dAlmeida, 2001)**. The disparity in size between the growth shooters and non-growth shooters is also accounted for by *archikotic* and *telikotic* growth phases with the archicotic phase weighing heavily on the distinction.

Identification of individual fish within a collection of the same species can be daunting since the nature of fish including their shape and body composition are designed to evade capture **(Zhang *et al*., 2012)**. The classification of fish based on temporal changes in length was fairly accurate when all fish were included (growth shooters and non-growth shooters). This shows that the full blend of population parameters in this case length **(Fréon, 1983)** is important in delineating the members of each cross within the population. In the non-growth shooter group, identification of individual along the line of cross is more difficult because there is some size uniformity. This is also true in the wild where cohorts of schooling fish have been observed to get lost within a school with reduced size variation **(Fréon, 1983)**. So as the class of progeny that are growth shooters delineated by cross reveal, size heterogeneity which was present in Cl×Cl and Ht×Ht judging from minimum and maximum values of length in weeks 1 and 8, can be proposed as the reason behind accuracy of discrimination among the growth shooters. When comparing discrimination between growth shooters and non-growth shooters, it is clear to see that a greater number of non-growth shooters were placed among growth shooters and this further shows the difficulty in classifying fish of fairly uniform size.

The agglomerative clustering of the crosses to depict their phylogeny shows that there was a clustering along maternal lines. This clearly shows a maternal influence on growth in length of the progeny **(Hagmayer *et al*., 2018; Heath *et al*., 1999)**. Maximum final length of the progeny was clearly divided along maternal lines such that there was similarity in values. In the chinook salmon, the maternal effect has been reported to affect offspring size in early ontogeny but declined with age until it ceased to exist **(Heath *et al*., 1999)**. This could also be the case for these crosses, but the duration of the current trial did not permit the monitoring of this effect.

The current research has shown that progeny from the crosses of *C. gariepinus* and *H. longifilis* can be grouped based on temporal progression in length. The delineation of the progeny into their respective crosses can be done using length information to a fair degree of accuracy. However, the ability to differentiate progeny according to the cross they came from declines with uniformity in size. The length of progeny as used to describe their growth have been given new nomenclature as *archikotic* growth phase (early growth), *telikotic* growth phase (late growth), *endiametic* (intermediate growth phase and *architelic* (initio-final) growth phase.

Conflict of interest

The authors have no conflicts of interest to declare.

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