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## RESEARCH ARTICLE

### CHARACTERIZATION OF THE MORPHOLOGY OF THE THREE STRAINS OF *OREOCHROMIS MACROCHIR* (BOULENGER, 1912) IN ZAMBIA

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#### ABSTRACT

The study was conducted at Misamfu Aquaculture Research Station in Zambia to evaluate the morphology of three *Oreochromis macrochir* populations from Chambeshi, Kafue and Luapula rivers for recommendation to the species genetic improvement programme, as a prerequisite before a genetic improvement programme is undertaken. Body measurements were collected on 20 adult specimens of each population to study morphological differences among the three strains. Principal component analysis (PCA) for the three strains indicated no significant difference ( $p=0.351$ ), with the first principal component (PC1), which is size, explained 59.7% of the variation while the second PC, which is the shape, explained 18.5 % of the variation. The variables that had high loadings on the second PC were PADC (80.7%), HED (73%) and VED (68.3%). Principal component analysis (PCA) of morphometric measurements between Chambeshi and Luapula indicated 75.2% of the variation was due to the first two components (PC1 [59%] and PC2 [16.2 %]). Analysis of variance on PC scores of PC2, whose shape, showed that there were significant differences ( $p=0.027$ ) in shape between the two strains. The PCA between Chambeshi and Kafue indicated 82% of the variation was due to the first two components (PC1 [58.7%] and PC2 [23.3 %]). Analysis of variance on PC score of PC2 showed that the two strains were not significantly different ( $p=0.979$ ) in shape. The PCA between Kafue and Luapula indicated 80.9% of the variation was due to the first two components (PC1 [61.8%] and PC2 [19.1%]). Analysis of variance on PC score of PC2 showed that the two strains were not significantly different ( $p=0.249$ ) in shape. The study concluded that the Chambeshi with Kafue and, Luapula with Kafue strains were not significantly different in the measured parameters, while Chambeshi and Luapula differed significantly on the horizontal eye diameter, vertical eye diameter and the cheek depth, and therefore the observed differences could be attributed to geographical separation.

#### INTRODUCTION

*Oreochromis macrochir* is one of the local fish species reared in Zambia by most small farmers across the country and most favoured due to its availability and is endemic to Luapula, Kafue and Chambeshi rivers (Gopalakrishnan, 1988, Kefi *et al.*, 2013; Hasimuna *et al.*, 2020; Maulu *et al.*, 2019; Hasimuna *et al.*, 2021). Several research efforts have been done to compare and assess its growth performances against other fish species but none has been done to compare the growth of Chambeshi, Kafue and Luapula strains.

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Zvavahera *et al.* (2018), Nsonga (2014) and Kefi *et al.* (2010) confirmed that *Oreochromis macrochir* could be reared by the extensive, semi-intensive and intensive farming system in aquaculture. Therefore, there was a need to carry out more detailed research in morphometrics to determine if they are morphometrically the same as a first step considered in strain evaluations studies since morphometric differences among fishes depict differences in growth rates as the body form is the origin and development of an organism (ontogeny); the reason why morphometrics is considered before a selective breeding programme is undertaken (Cadran, 2005). The use of colour or other external features (Phenotypes) of differentiating fish stocks has been used more frequently rather than morphometric measurement (Creech, 1992; Mamuris *et al.*, 1998; Bronte *et al.*, 1999; Hockaday *et al.*, 2000). Fishes in most cases show much greater differences in morphological parameters either within the same population or between

populations as compared to other organisms, hence making them prone to environmental factors that induce morphological differences (Dunham *et al.*, 1979; Allendorf, 1988; Thompson, 1991; Wimberger, 1992). Morphometrics is the evaluation of the body size and shape of an organism (Costa *et al.*, 2006). Size in morphometrics is defined as the geometric mean of all variables and is widely regarded as a fundamental aspect of any organism's biology that has a bearing on the behaviour including its anatomy and physiology (Jungers, 1985a; McGowan, 1994). The goal of many comparative studies is to assess similarities or differences among taxa after size is taken into account or factored out. Mosimann (1970) observed that shape in organisms is an intrinsic factor of an organism and not the changing function of different comparative sets and, that shape correlates with changes in size according to Mosimann (1987). Morphometric evaluation is mainly done on living organisms as well as in analyzing body shape mutations due to changes in form as a result of environmental factors that influences shape formation and, it also assesses co-variances as well for measuring genetic variables in shapes.

Morphometrics is also employed in quantifying traits that come about due to changes of evolution through detecting changes in body parameters that affect the shape and evaluate evolutionary similarity among close relations (Costa *et al.*, 2006). The major purpose of morphometrics assessment is to prove the statistical hypotheses on the parameters that influence the shape. After the invention of principal components analysis (truss method) which is a multivariate statistic in evaluating the fish morphology by Hanken (1986), it became much easier to evaluate fish morphometrics. Morphometrics study has helped to better analyze measurements of parameters of shape descriptions by allowing more detailed comparisons between and among shapes. It enables scientists to describe shapes that are complex in nature. Morphometrics guides in quantifying an aspect of shape descriptions through more detailed analysis in comparisons of between or among species shapes. It enables scientists to describe shapes that are complex in a rigorous fashion and allows numerical comparison between forms (Costa *et al.*, 2006). The use of advanced technology in analyzing morphometrics has helped to easily detect differences in shape as well as to isolate shape from size variation according to Costa *et al.* (2006).

**MATERIALS AND METHODS**

**Study Area:** The study was conducted at Misamfu Aquaculture Research Station (MARS) in Kasama district of the Northern Province of Zambia involving three populations of *Oreochromis macrochir* drawn from Chambeshi, Luapula and Kafue rivers.



Figure 1 Map of Africa Source: Chameleon web services (2021)

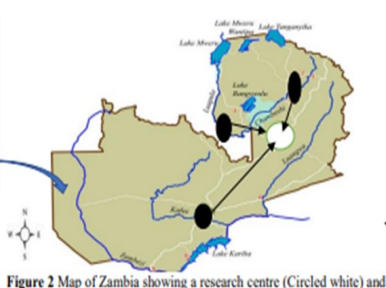


Figure 2 Map of Zambia showing a research centre (Circled white) and Brood stock site collection in black spots Source: Zambia tourism (2018)

Twenty fish specimens from each strain (N=60) were collected from each site using a nylon monofilament gill net (2.5 inches) in November, 2019. The monofilament gill nets were set by the fisher folks at the selected sites and hauled after two hours. After capture, the standard length (SL) of approximately 170 mm and weighing approximately 200g of each fish was measured and the specimen tagged for morphometrics characterization purposes, which were then anaesthetized with clove powder for morphometric analysis. Thereafter, all the measured fish samples were preserved in 10% formalin and later stored permanently in 70% ethanol for future reference at Misamfu Fisheries Research Station Laboratory. All measurements were made from the left side of the fish following Barel *et al.* (1977), Stauffer (1991; 1994) and Stauffer and Koning (2006) (Figure 3). A vernier calliper was used to take and make morphometric measurements.

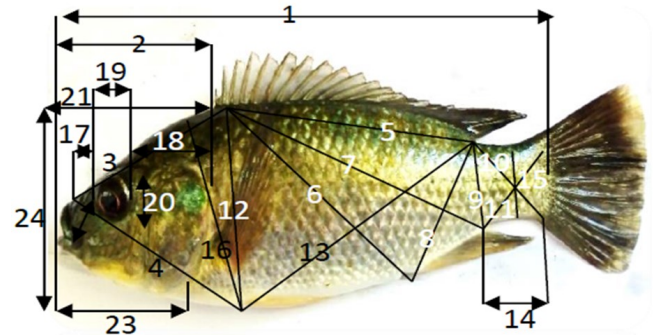


Figure 3. Morphometrics measurements on *Oreochromis macrochir*

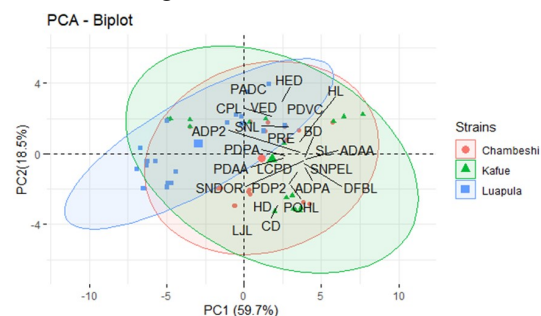
The parameters for morphometric analysis were determined on the left side of the fish with its body belly facing the recorder

**Data analysis**

Morphometric information collected was analyzed using principal components analysis (PCA), on second principal component (PC 2) which represents shape to test for any significant differences at an alpha level of 0.05, on all the three strains and later a paired comparison was performed between the strains for any significant differences. R statistical software, R version 3.5.1, R (R Core Team, 2018) and two R packages; FactoMineR (Le *et al.*, 2008); Factoextra (Kassambara and Mundt 2017) was used in the analysis.

**RESULTS**

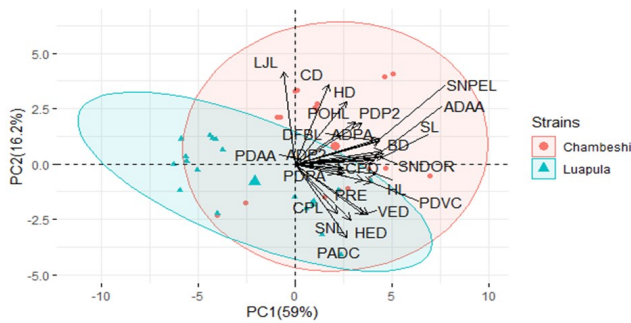
**Morphological variation among the three strains of *Oreochromis macrochir*:** The character variation measured by loadings for the 24 morphometric characters on the three strains is shown in Figure 4.



Key: TL - Total length; SL - Standard Length; HL - Head Length; SNDOR - Snout dorsal fin origin; DFBL - Dorsal fin base length; ADAA - Anterior dorsal anterior anal; ADPA - Anterior dorsal posterior anal; PDAA - Posterior dorsal anterior anal; PDPA - Posterior dorsal posterior anal; PDVC - Posterior dorsal-ventral caudal; PADC - Posterior anal dorsal caudal; ADP2 - Anterior dorsal pelvic-fin origin; PDP2 - Posterior dorsal pelvic-fin origin; CPL - Caudal Peduncle Length; LCPD - Least caudal peduncle depth; BD - Body depth; SNL - Snout Length; POHL - Postorbital head length; HED - Horizontal eye diameter; VED - Vertical eye diameter; PRE - Preorbital length; CD - Cheek depth; LLL - Lower jaw length; HD - Head Depth

Figure 4. Morphological variation among the three strains of *Oreochromis macrochir*

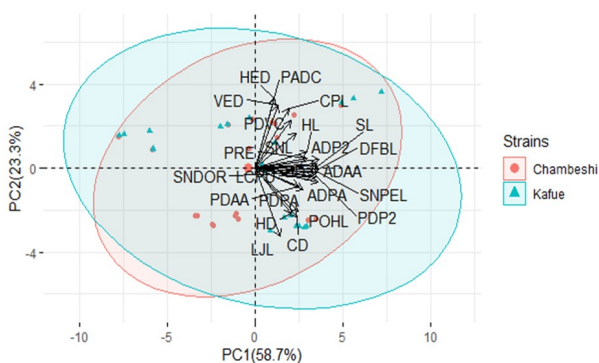
The first principal component (PC1), which is size, explained 59.7% of the variation while the second PC, which is about the shape, explained 18.5 % of the variation. The variables that had high loadings on the second PC were PADC (80.7%), HED (73%) and VED (68.3%). There were no significant differences ( $p=0.351$ ) among the three strains on scores of PC2.



Key: TL - Total length; SL - Standard Length; HL - Head Length; SNDOR - Snout dorsal fin origin; DFBL - Dorsal fin base length; ADAA - Anterior dorsal anterior anal; ADPA - Anterior dorsal posterior anal; PDAA - Posterior dorsal anterior anal; PDPA - Posterior dorsal posterior anal; PDVC - Posterior dorsal-ventral caudal; PADC - Posterior anal dorsal caudal; ADP2 - Anterior dorsal pelvic-fin origin; PDP2 - Posterior dorsal pelvic-fin origin; CPL - Caudal Peduncle Length; LCPD - Least caudal peduncle depth; BD - Body depth; SNL - Snout Length; POHL - Postorbital head length; HED - Horizontal eye diameter; VED - Vertical eye diameter; PRE - Preorbital length; CD - Cheek depth; LYL - Lower jaw length; HD - Head Depth

**Figure 5. Morphological variation between Chambeshi strain and Luapula strain of Oreochromis macrochir**

**Morphological variation between Chambeshi and Luapula strains:** The character variation measured by loadings for the 24 morphometric characters on the Chambeshi and Luapula strains of *Oreochromis macrochir* is shown in Figure 2. The first PC, which is size, explained 59% of the variation while the second PC, which is about shape, explained 16.2 % of the variation. The variables that had high loadings on the second PC were LYL (92.5%), CD (72.1%) and HD (59.5%). There were significant differences ( $p=0.027$ ) between the two strains on scores of PC2. For LYL, the Chambeshi strain had a higher length than the Luapula strain. For CD, the Chambeshi strain had a higher length than the Luapula strain. For HD, the Chambeshi strain had a higher length than the Luapula strain.



Key: TL - Total length; SL - Standard Length; HL - Head Length; SNDOR - Snout dorsal fin origin; DFBL - Dorsal fin base length; ADAA - Anterior dorsal anterior anal; ADPA - Anterior dorsal posterior anal; PDAA - Posterior dorsal anterior anal; PDPA - Posterior dorsal posterior anal; PDVC - Posterior dorsal-ventral caudal; PADC - Posterior anal dorsal caudal; ADP2 - Anterior dorsal pelvic-fin origin; PDP2 - Posterior dorsal pelvic-fin origin; CPL - Caudal Peduncle Length; LCPD - Least caudal peduncle depth; BD - Body depth; SNL - Snout Length; POHL - Postorbital head length; HED - Horizontal eye diameter; VED - Vertical eye diameter; PRE - Preorbital length; CD - Cheek depth; LYL - Lower jaw length; HD - Head Depth

**Figure 7. Morphological variation between Kafue strain and Luapula strain of Oreochromis macrochir**

**Morphological variation between Chambeshi and Kafue strains:** The character variation measured by loadings for the 24 morphometric characters on the Chambeshi and Kafue strains of *Oreochromis macrochir* is shown in Figure 6.

The first PC, which is size, explained 58.7% of the variation while the second PC, which is about the shape, explained 23.3 % of the variation. The variables that had high loadings on the second PC were PADC (80.7%), HED (73%) and VED (68.3%). There were no significant differences ( $p=0.979$ ) between the two strains on scores of PC2.

**Morphological variation between Kafue and Luapula strains:** The character variation measured by loadings for the 24 morphometric characters on the Kafue and Luapula strains of *Oreochromis macrochir* is shown in Figure 7. The first PC, which is size, explained 61.8% of the variation while the second PC, which is about shape, explained 19.1% of the variation. The variables that had high loadings on the second PC were PADC (80.7%), HED (73%), and VED (68.3%). There were no significant differences ( $p=0.249$ ) between the two strains on scores of PC2.

**DISCUSSION**

The three-strain comparison (Chambeshi, Luapula and Kafue) and the pairwise comparison for (Chambeshi with Kafue) and (Kafue with Luapula) did not vary in size and as well as in shape indicating that they are morphometrically the same. The current study attributes its results to the similarity in ecological regions, hence the closeness in morphometrics is due to being in the same geographical area as they are in the same watershed. Costa (2007) and Hanif *et al.* (2019) reported that close similarities among fish stocks may be the consequence of habitat characteristics and possible homogenous environmental factors such as the trophic ecology. The differences obtained in the pairwise study between Chambeshi with Luapula on morphology can be attributed to genetic differences and to some extent the ecological environment such as feed type. The Chambeshi River rises as a stream from the northeast mountains of Zambia on an elevation above sea level of 1,760 metres, then it flows for 480 km into Lake Bangweulu. The water then flows out of the Lake as the Luapula River. This means that the Luapula River drains Lake Bangweulu and its swamps into which flows the Chambeshi river, the main reason as to why morphometrically the Chambeshi and Luapula fish were significantly different as they do not share the same geographical area.

According to Barlow (1961) and Swain and Foote (1999) indicated that the morphology of fish is influenced by the interaction of genetic and environmental parameters and morphology differences appear as the fish grows and might change depending on the location. Similarly, the current study has observed the same. Hanif *et al.* (2019) indicated that difference in the head region implies that they differ in the feeding habit whilst differences in the body region implies that they differ in locomotion which can be attributed to the water current. Robinson and Wilson (1996) observed that phenotypic plasticity is a result of genetic variations. Hanif *et al.* (2019); Mir *et al.* (2012) observed that differences could be associated with unpredictable aquatic circumstances such as temperature, turbidity, salinity, alkalinity and water current pattern. Hossain (2010) found that variations are a result of the migration of local fish and environmental parameters. Yamamoto *et al.*, (2006) described the variation to be a result of geographical isolation which produces morphological changes. Therefore, the study concurs with these findings observed by previous researchers, as the reason for such differences in some morphometric parameters between Chambeshi and Luapula strain.

The results of the current study are also similar to Hossen *et al.* (2017) study on morphology among three groups of Tilapia fish picked four different locations, and these differences were attributed to environmental parameters. Amarasinghe *et al.* (1983) and Chandrasoma *et al.* (1986) in Sri Lanka, indicated that limnological factors of water bodies vary from one location to the other and, that could have influenced their results. The current study concurs with previous studies conducted and further attributes the difference to geographical locations. Valentin *et al.*, (2014) reported that morphometric differences in fishes depict differences in growth rates as the body form is the origin and development of an organism (ontogeny) the reason why morphometrics is considered before a selective breeding programme is undertaken. Creech (1992), Mamuris *et al.* (1998), Bronte *et al.* (1999) and Hockaday *et al.* (2000) indicated that phenotypic difference in morphometric evaluation is the commonly used method to describe and define fish stocks. Fishes in most cases show much greater differences in morphological parameters either within the same population or between populations as compared to other organisms, hence making them prone to environmental factors that induce morphological difference which is seen in different environments, pertaining to feeding habits and food availability (Dunham *et al.*, 1979; Allendorf, 1988; Thompson, 1991; Wimberger, 1992).

## CONCLUSION

The present study observed that the studied *Oreochromis macrochir* strains from the three regions are similar in most morphometric characters with only Chambeshi with Luapula showing differences between them, hence this study provides basic information on the morphometric characterization of *Oreochromis macrochir* populations in the three river systems. The study has shown that the techniques used by Stauffer and Konings (2006) of analyzing the 24 characters on the fish body in morphometric differentiation, can effectively be applied in assessing the variation of stocks within a species in freshwater habitats.

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