

**MORPHOMETRIC VARIATIONS AMONG *OP SARIDIUM MICROLEPIS*
(GÜNTHER, 1864) FROM LAKE MALAŴI MIGRATING TO DIFFERENT RIVERS
FOR BREEDING**

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ABSTRACT

Fish is an important resource in Malawi as a source of food for the majority as it provides affordable source of dietary animal protein as well as income. A number of fish species in the Malawi water bodies have their population dwindling. One of the species under serious threat of extinction is the *Opsaridium microlepis* - a potamodromous fish species that migrate to the rivers during its spawning period and its management seems a nightmare. A number of studies reveal contrasting results on genetic makeup and morphological aspect of this fish species. With changes in the ecosystems of the rivers connecting Lake Malawi, coupled with absence of strong management measures in the major rivers adjoining the lake, problems have arisen in the conservation of potamodromous fish species. This necessitated the present study to investigate if the morphological features of stocks of *O. microlepis* are the same or not and if they have changed to adapt to changes in the ecosystems. One hundred and eleven *O. microlepis* fish samples were collected from Linthipe River (48), Bua River (59) and North Rumphu River (4) monthly from March to August 2020 using trawled and static gillnets. Twenty-four morphometric characteristics were measured to determine if any morphological differences existed among the fish samples from the three rivers. Principal component analysis (PCA) was used to compare morphology of the fish. Results of the study showed no significant morphological differences among stocks from the three rivers, implying that *O. microlepis* in these rivers belong to same stock morphologically. The study reveals that the species do not differ morphologically even though they migrate to different rivers for breeding. The study further notes that numerous activities taking place along the tributary rivers (as observed during the study) such as modification of fishing gears as well as fishing methods and the deterioration of the spawning grounds due to siltation from soil erosion caused by deforestation and agriculture, are putting the potamodromous fish species such as *O. microlepis* under serious threat. The study recommends that the populations of *O. microlepis* from the rivers can be managed equally since they are morphologically similar. Adopting uniform catchment management and sustainable exploitation of *O. microlepis* (such as regulations on mesh sizes and fishing methods, closing the rivers from fishing activities during spawning period, river bank and catchment management and restoration) with the aim of conserving the stocks from further overexploitation in these rivers is recommended so that the communities and the people at large continue to utilize the resource sustainably and at the same time, sustaining their livelihood.

Key words: Potamodromous, Linthipe River, Bua River, North Rumphu River, principal component analysis



INTRODUCTION

Fish morphometrics are studied extensively in fisheries science [1]. Variations in fish morphology and body shape have often been described subjectively and qualitatively [2]. Such kind of morphological variations which form the basis of the apparent biological diversity of fishes [2], are due to genetic divergence and/or phenotypic plasticity [3, 4], and may lead to adaptive radiation [5-8]. The landmark based morphometric traits have lately gained popularity in documentation and description of numerous taxonomic groups [9,10]. However, size must be considered as a contingent source of variability in morphometrics, since it is associated with individual growth and the aim of such studies is usually focused on shape that must be size-free [11]. Body shape has been recognized recently as the most essential and integrative aspect of an organism's phenotype [12]. In addition to body size and sex, diet and resource utilization affect the fish morphology, in respect to particular dietary items which can induce morphological change within or among populations, and all of these factors can be complicated by environmental variation with phenotypic plasticity [6].

The *Opsaridium microlepis* (Günther, 1864) also called the lake salmon is a freshwater African fish species endemic to Lake Malaŵi [13], in the Cyprinidae family found in Malaŵi, Mozambique, and Tanzania. It is a potamodromous fish and its natural habitats are rivers and freshwater lakes [14]. As a potamodromous fish, it moves and completes its life cycle entirely within freshwater. It is a silvery fish which resembles trout of the family Salmonidae. They are the largest in the *Opsaridium* group and can grow up to 4 kg in weight [15] and 70 cm in total length [16]. In Malawi, during the rainy season, the adult fish migrate up the tributary rivers from the lake to spawn, and this mainly takes place at night in shallow, well-oxygenated, flowing waters over gravel substrates with no silt. In Tanzania, *O. microlepis* migrates to the rivers (such as Ruhuhu River) during the dry season [17]. A fish such as *O. microlepis* rely on multiple habitats and open passages to migration destinations. The conservation of migratory fishes hereby demands complex consideration to enhance the probability of completion of all life stages [18].

However, the spawning rivers on the Tanzania side for this species have significantly been affected such a case being the Ruhuhu River where it is heavily exploited around the mouth and in the upper reaches of the river itself [17]. The absence of strong management measures in the big rivers of Malaŵi where fish species migrate to spawn, has also exacerbated the problems in the conservation



of potamodromous fishes and other species. This, therefore, necessitated the present study to investigate if the stocks of *O. microlepis* in these rivers are morphologically the same or not and hence recommend measures to conserve the stocks. Anthropogenic activities, intentional as well as unintentional, have affected Lake Malawi and its ecosystem. Siltation from agricultural runoff and increased land use is destroying the lake habitats and breeding areas [19-21]. Timing fishing of migratory fish especially the *O. microlepis* in the rivers joining L. Malaŵi during their breeding season poses a serious threat to the species' recruitment [22,]. The *O. microlepis* was listed in the Red List of Threatened Species of the world by the International Union for Conservation of Nature (IUCN) [15]. In 2018, *O. microlepis* again was listed by the IUCN Red List as seriously decreasing due to serious overfishing, pollution from agricultural effluents, dams and water management/use due to natural system modifications and also droughts due to climate change and severe weather. As a way to enhance biological productivity of *O. microlepis* within the rivers they migrate to for breeding, there is need to validate similarities of their stock.

Several methods have been employed in species identification and stock structure analysis including the use of ecological studies, tagging, distribution of parasites, physiological and behavioural aspects, morphometrics and meristics, calcified structures, cytogenetics, immunogenetics, blood pigments, allozyme electrophoresis and nucleic acid analysis [23-26]. Morphological characters are important in fish species identification while growth is important in the evolutionary persistence of a fish species in the habitat, their assessments are important in evaluating evolutionary changes in a population [27]. Anatomical characters have traditionally been used in fisheries biology to describe geographic variation in a wide variety of exploited species [23]. In this regard, it is worthwhile stating that data on species identification and stock structure are useful in the context of management and conservation of fish species only if such information is fully incorporated into stock assessment. Morphometrics include the analysis of body shape, or the shape of particular morphological features of various body dimensions or parts and indeed morphometric expression is under the simultaneous control of genetic and environmental factors [28, 29]. In the current study, we hypothesized that there are no differences in the morphological features of *Opsaridium microlepis* fish from the three tributary rivers.



MATERIALS AND METHODS

Study sites

Fish samples of *O. microlepis* were collected from Bua, Linthipe and North Rumphu Rivers (Figure 1).

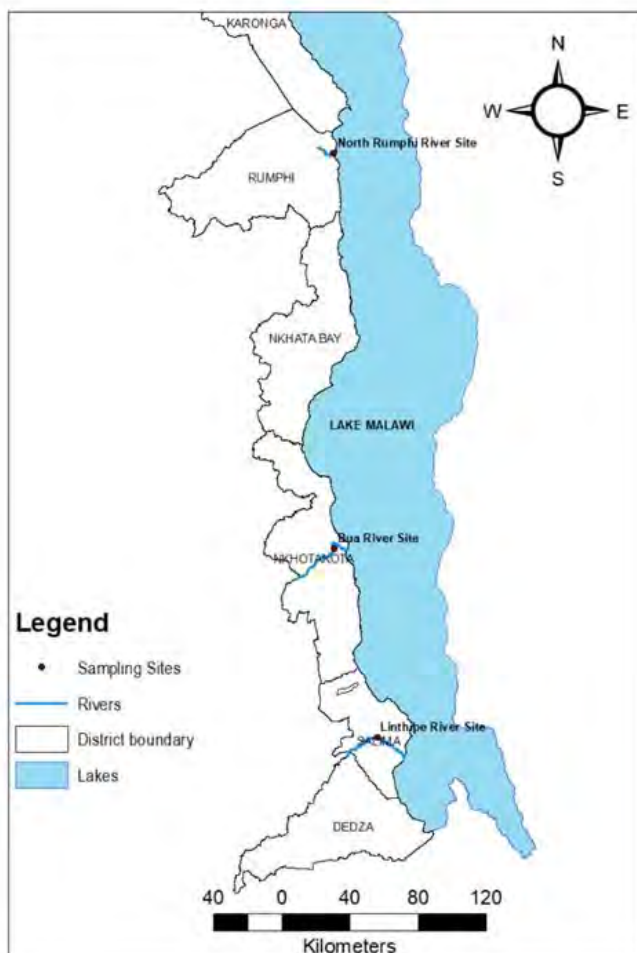


Figure 1: Sampling rivers of *O. microlepis*

Bua River is located in the central part of Malaŵi and it is one of the tributaries of L. Malaŵi where *O. microlepis* migrate to spawn during the breeding period. There is overfishing and use of weirs in Bua River as it flows out of Nkhotakota Game Reserve, which has conservation measures for *O. microlepis* unlike in the lower part of the river. The river banks are heavily cultivated and this is increasing the rate of siltation of the river and impacting on the habitats of the fish both in the river and L. Malawi.

On the other hand, Linthipe River is a major spawning river of *O. microlepis* in the central part of Malawi [30], yet it is heavily affected from farming systems [31] taking place along the river banks. Furthermore, development activities exert land pressure which is significantly affecting the species' productivity. It was noted that *O. microlepis* is facing serious problems from both fishing pressure and environmental degradation [30-32]. It is therefore important to come up with a management strategy for conservation of *O. microlepis*.

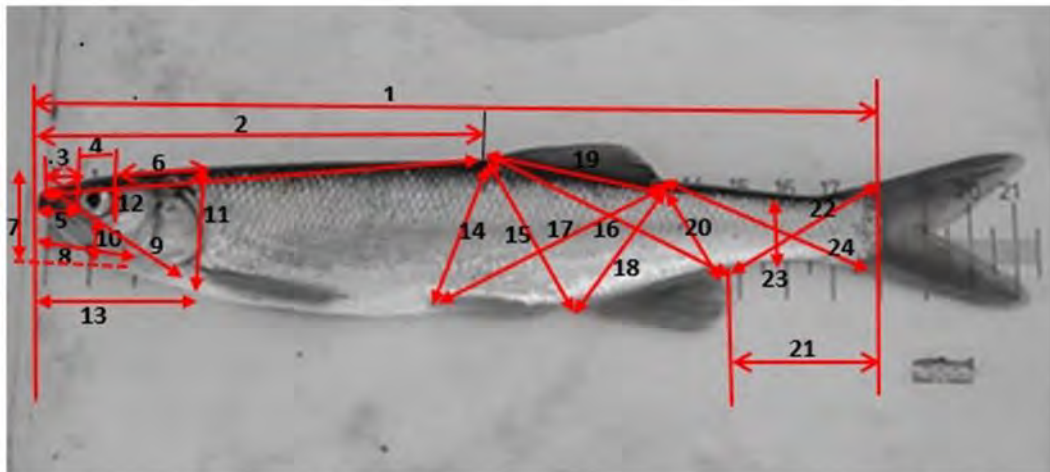
The North Rumphi River is located in the northern part of Malawi and passes through escarpments and the Wongwe Falls. Most of the catchments of North Rumphi River are covered with vegetation and rocks such that they are well protected apart from the lower reaches which are now being degraded due to agricultural activities taking place as the river enters the lake (observations during sampling).

Fish Sampling

The sampling was conducted from March to August 2020 and one hundred eleven (111) *O. microlepis* specimens were collected from Linthipe, Bua and North Rumphi Rivers (the distance between Linthipe River and Bua River is about 122 km; Bua River and North Rumphi River about 330; between Linthipe and North Rumphi about 450 km) using static gillnets which were set across the rivers from the selected sites and drifting gillnets (all of them with mesh sizes of 3 to 4.5 inch), from a stretch of about 3 km down to the river mouths. For the static gillnetting, the nets were set at 17:00 hours and retrieved at 06:00 hours the following day. For the drifting gillnets, fishing was done both at night and day time to catch the fish. There was variation in number of samples within the months (Table 1) because fish could not be found during the sampling of fish in some of the months.

Three taxonomists were assigned to identify *O. microlepis* fish in this study. Fish samples were taken immediately once the fishers retrieved the nets to preserve their freshness. The fish were preserved in ice in heavy duty cooler boxes and taken to the laboratory at Lilongwe University of Agriculture and Natural Resources (LUANAR) for morphometric measurements. A vernier caliper (KANON, KSM - 20 200 x 0.05 mm) was used for measurements, following the procedure described by Konings [33]. Fish were also weighed to the nearest gram (± 0.01 g) using an Adam CKT 16 (13667) electronic weighing balance. The 24 morphometric characteristics were measured (all in mm) and are shown in Figure 2 and Table 2.





KEY: 1. SL, 2. SNDOR, 3. PRE, 4. HED, 5. SNL, 6. POHL, 7. HD, 8. L JL, 9. SNPEL, 10. CD, 11. BD, 12. VED, 13. HL, 14. APD2, 15. ADAA, 16. ADPA, 17. PDP2, 18. PDAA, 19. DFBL, 20. PDPA, 21. CPL, 22. PAD, 23. LCPD, 24. PDVC

Figure 2: Morphometric measurements taken on the left side of *O. microlepis*

Data analysis

Morphometric measurements were tested for adequacy and suitability using Kaiser-Meyer-Olkin (KMO) Test and Bartlett's test of sphericity in Statistical Package for Social Scientists (SPSS) version 20.7 computer package. The KMO measures the sampling adequacy, for example, if the sample data are adequate or not [34] to assess the factor structure [35]. A value of 0.5 (value for KMO) is recommended as minimum (barely accepted), values between 0.7-0.8 acceptable, and values above 0.9 are excellent [36]. Bartlett's test helps to identify the strength of the relationship between variables [34].

The data on morphometry was for example, collated in Excel and principal component analysis (PCA) was performed in R Software package, version 3.9 to determine the morphometrical differences among the groups of fish from Bua, Linthipe and North Rumphu Rivers. The PCA is a multivariate technique useful in determining latent factors that describe size and shape variations. It uses matrix algebra to model a correlation matrix as a set of orthogonal (perpendicular) axes, or principal components [37]. Each PC axis corresponds to an eigenvector. The eigenvalue describes the variance accounted for by the corresponding axis. The first principal component (PC1) is assumed to be a general representation of fish size if the loadings are the same sign and similar in magnitude [38-39]. In practice, the PC1 of morphometrics is interpreted as a size axis when variable loadings are similar in magnitude and sign [38-39], and the second principal components (PC2) are interpreted as shape variables [8, 39]. Descriptive statistics were performed on

the morphometric measurements and ANOVA test done to test significant differences in the morphological measurements.

RESULTS AND DISCUSSION

The appropriateness of factor analysis was supported by Bartlett's test of sphericity, an indicator of the strength of relationship among variables. It was found that the results were significant ($\chi^2 = 5201.06$) (Table 3). The KMO measure of sampling adequacy yielded a value of 0.97.

The KMO value for the morphometry data was found to be over 0.9 and values for KMO values above 0.9 are excellent. The measured morphometric data in this study was therefore adequate for such kind of test. The KMO result herein therefore indicates that the sample size was large enough to assess the factor structure (Table 3). With the value above 0.9, all the samples were adequate for Factor Analysis. Bartlett's test helps to identify the strength of the relationship between variables [34]. Bartlett's test of Sphericity shows that the correlation matrix possesses significant information.

The analysis of variance (ANOVA) test showed no significant differences in the morphological characteristics among the *O. microlepis* from the three rivers but there were variations in standard length (SL) (ranging from 210.0-530.0 mm), snout to dorsal fin origin (SNDOR) (ranging from 96.00-262.70 mm) and snout to pelvic-fin origin (SNPEL) (ranging from 32.20-260.50 mm) of individuals sampled from the three water bodies (Table 4).

Descriptive statistics showed variations in standard length (SL) (ranging from 210.0-530.0 mm), snout to dorsal fin origin (SNDOR) (ranging from 96.00-262.70 mm) and snout to pelvic-fin origin (SNPEL) (ranging from 32.20-260.50 mm) of individuals sampled from the three water bodies (Table 4). These variations were suggested to be from differences in ecological aspects in the different rivers but are found not to bring any significant differences in terms of stock identity. The mean values of most morphometric characteristics were similar among the fish of Bua, Linthipe and North Rumpi Rivers. The following morphological characteristics; standard length (SL), head length (HL), snout to dorsal-fin origin (SNDOR), snout to pelvic-fin origin (SNPEL), dorsal-fin base length (DFBL), anterior dorsal to anterior anal (ADAA), anterior dorsal to posterior anal (ADPA), posterior dorsal to anterior anal (PDAA), posterior dorsal to posterior anal (PDPA), posterior dorsal to ventral caudal (PDVC), posterior anal to dorsal caudal (PADC), anterior dorsal to pelvic-fin origin (ADP2), posterior dorsal to pelvic-fin origin



(PDP2), caudal-peduncle length (CPL), least caudal-peduncle depth (LCPD), body length (BD), postorbital head length (POHL), vertical eye diameter (VED), preorbital length (PRE), cheek depth (CD), lower-jaw length (LJL) and head depth (HD) contributed greatly to the first PC (size) while horizontal eye diameter (HED) and snout length (SNL) contributed the least (63.16% and 23.45%, respectively).

Principal component 1 which is size, showed a variance of 58.2% while PC2 which is shape, showed a variance of 15.61%. In PC2, which is shape, it was only the horizontal eye diameter (HED) which has the highest variation (91.50%) while the rest have no significant differences ($p > 0.05$). This entails that in terms of shape, the *O. microlepis* from the 3 rivers only vary in the horizontal eye diameter and not the rest of the other parameters and this could be explained that it could be water quality issues such as turbidity that could make the fish in the different rivers such as Linthipe to have differences in the eye diameter as the fish tries to adjust for visibility issues. The first principal component (PC1), which is size, explained 81.68% of the variation while the second PC, which is shape, explained 4.24% of the variation. This study therefore reveals that the potamodromous *O. microlepis* in L. Malawi is of the same stock despite migrating to different rivers for spawning. This entails that management measures targeting this species (such as mesh sizes restriction etc.) can be applied uniformly across the rivers to make sure the species is conserved from further overexploitation.

The results of pairwise comparison of the stocks from the 3 rivers show that horizontal eye diameter strongly and positively contributed highly to the principal component 2 (shape) among the fish groups from the three rivers (Figure 3). Two samples were found to be outside the Bua suggesting that they are morphologically different from Bua River and similar to North Rumphu River in terms of shape.

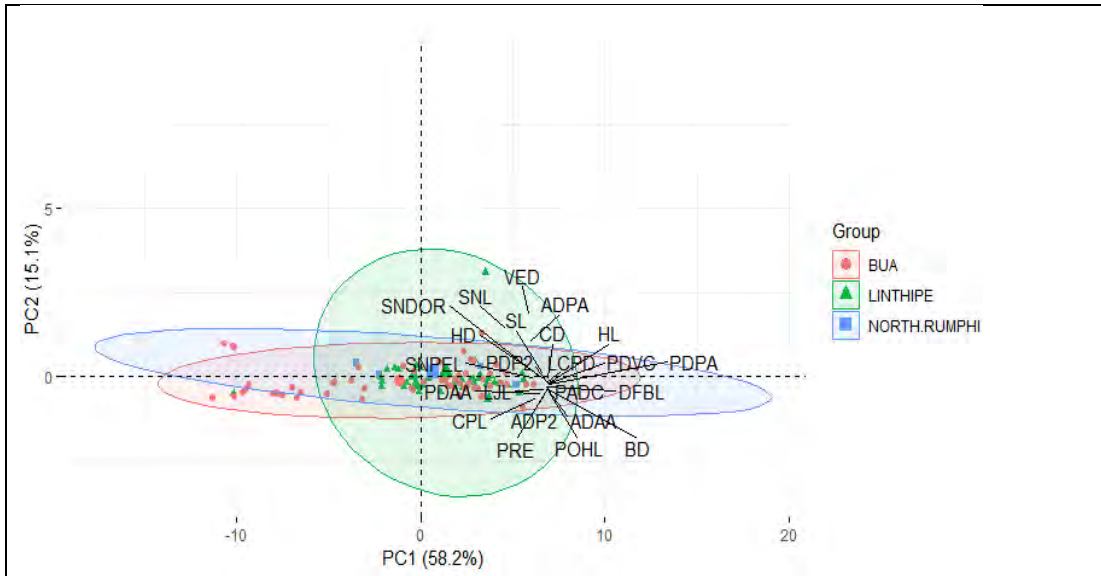


Figure 3: PCA comparisons of *O. microlepis* fish stocks from Linthiipe, Bua and North Rumpi Rivers

This means that the morphometric attributes of size were not statistically different ($p > 0.05$) for the *O. microlepis* fish from the three rivers, as such they are of the same stocks. All the other attributes were weak and contributed lowly to both principal components 1 and 2 (size and shape respectively). A pairwise PCA comparison for Bua and Linthiipe Rivers also shows a strong and high contribution of horizontal eye diameter to principal component 2 (shape) compared to all the other 23 attributes for the *O. microlepis* fish from these two rivers (Figure 4).

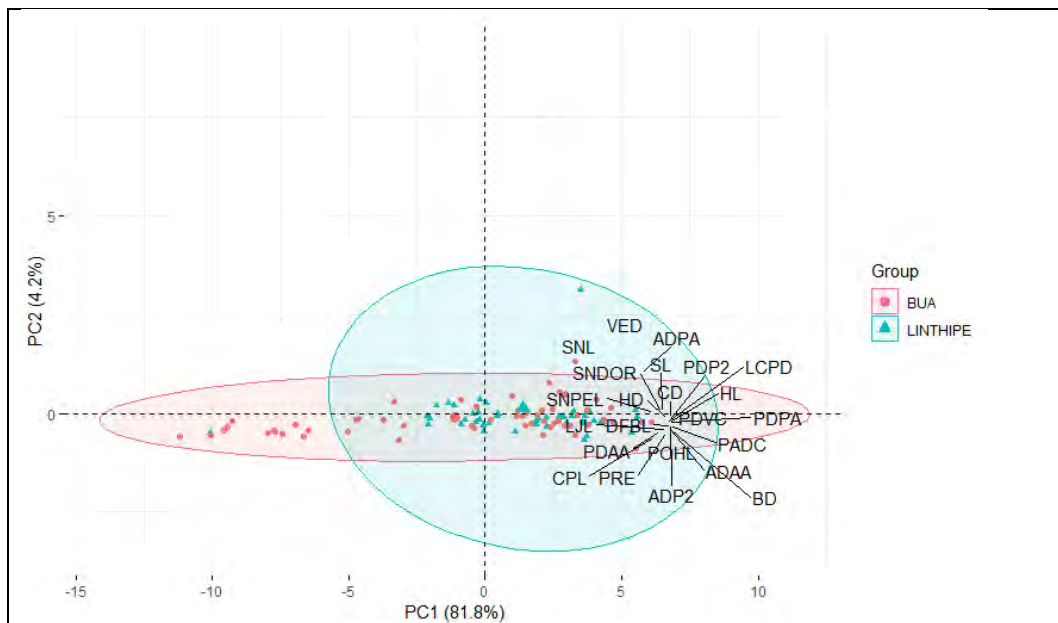


Figure 4: Pairwise PCA comparisons of *O. microlepis* fish from Linthiipe and Bua Rivers

The morphological aspects of the fish from these two rivers are closely related perhaps explaining the fact that these rivers are approximately 50 km away from each other. These results indicate that the *O. microlepis* from Bua and Linthipe are morphologically the same.

A pairwise PCA comparison for Bua and Linthipe Rivers also shows a strong and high contribution of horizontal eye diameter (HED) to principal component 2 (shape) compared to all the other 23 morphometric attributes for the *O. microlepis* fish from these two rivers (Figure 4).

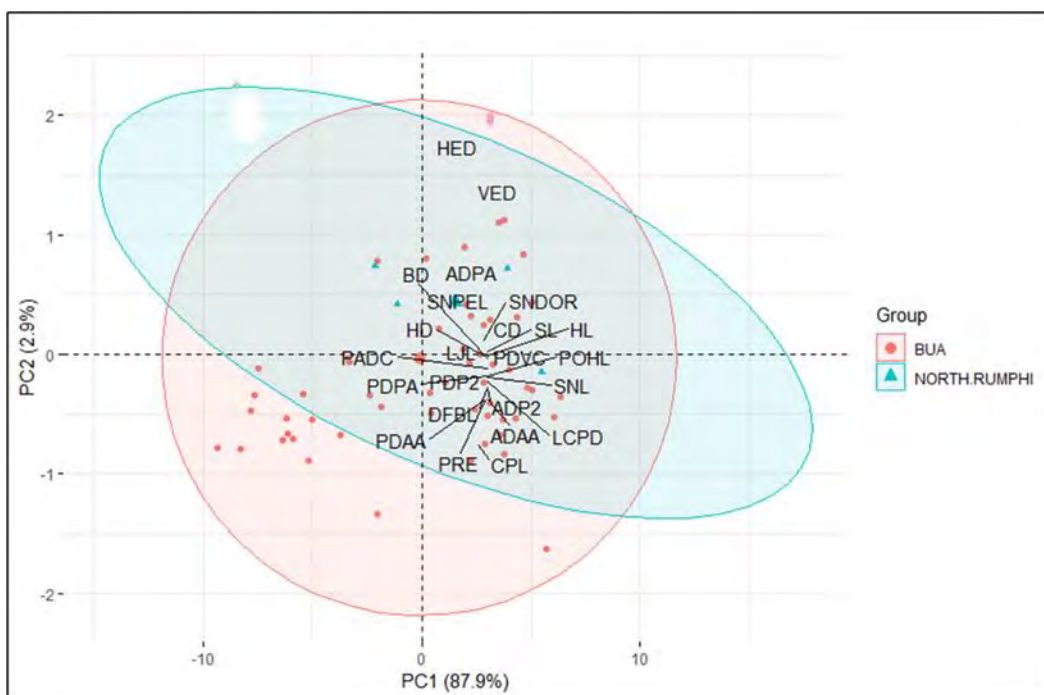


Figure 5: Pairwise PCA comparisons of *O. microlepis* fish from Bua and North Rumphi Rivers

The pairwise PCA of *O. microlepis* from Linthipe and North Rumphi (Figure 6) gives a similar trend as of those from Bua and North Rumphi comparison with morphometrical aspects closely to each other but differing in the components that contribute to PC2 (shape).

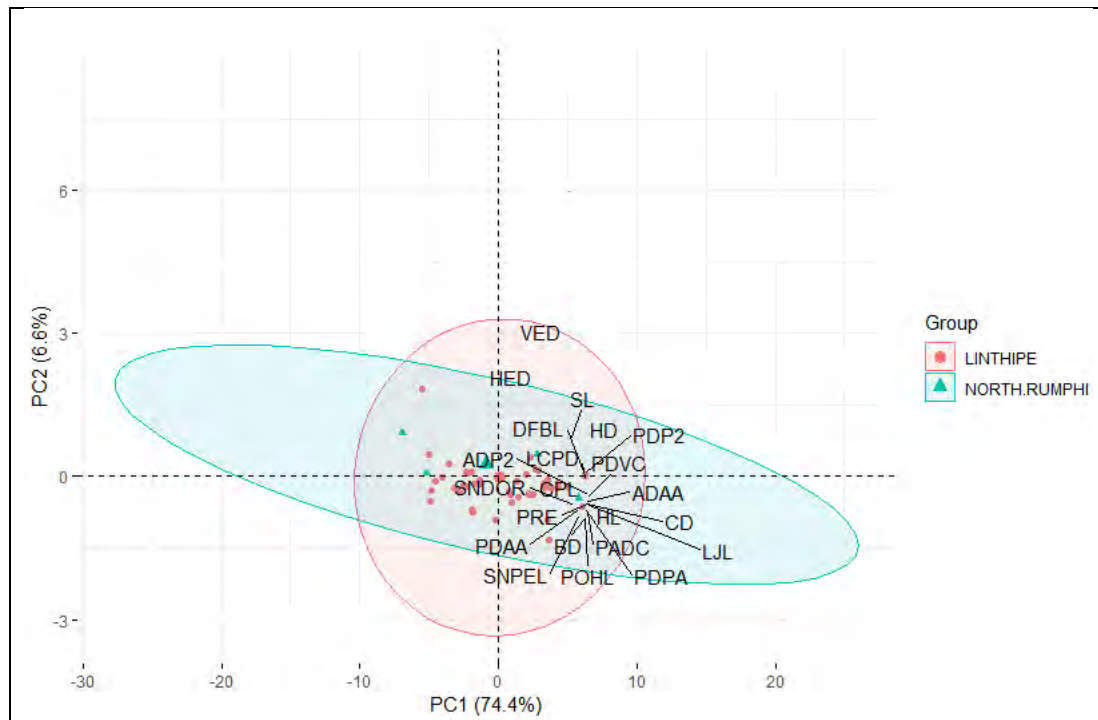


Figure 6: Pairwise PCA comparisons of *O. microlepis* fish from Linthi and North Rumpi Rivers

From Table 5, all the morphometric landmarks measured for the *O. microlepis* for PC1 contributed highly and positively for all the 3 rivers, indicating no significant differences among the fish from the rivers ($p > 0.05$). Consequently, on PC2, the shape attributes contributed weakly and negatively for all the 3 rivers, again signifying no significant differences in shape for the *O. microlepis* from the three sampled rivers.

The pairwise PCA of *O. microlepis* from Linthi and North Rumpi (Figure 6) gives a similar trend as of those from Bua and North Rumpi Rivers comparison with morphometrical aspects closely to each other but differing in the components that contribute to PC2 (shape). In this regard, snout length and anterior dorsal to anterior anal contributing strongly and highly to principal component two (PC2) which is shape. May it be mentioned that these two rivers are located at a distance of about 400 km from each other.

With a total variance of 73.3% (58.2% by PC1, and 15.61% by PC2) explained for the first two axes it simply means that overall, the PCA showed no significant differences in morphology of the *O. microlepis* from Bua, Linthi and North Rumpi Rivers. This is also explained, but interestingly, by pairwise PCA of *O. microlepis* from Bua and North Rumpi in Figure 5 and give another dimension of

the relationship between the *O. microlepis* fish from these two rivers. Even though the PCA results indicate no significant differences in the morphometric characteristics of the two stocks from Bua and North Rumphi, the morphological aspects tend to scatter around the biplot of the PCA, with horizontal eye diameter (HED) and vertical eye diameter (VED) contributing strongly and highly on PC2. Perhaps this might be due to the fact that these two rivers are located at a distance of more than 300 km away from each other. This phenomenon is depicted in the PCA pairwise for Bua and North Rumphi as well as Bua and Linthipe where PC 2, which is shape, where there is no significant difference but still a few specimens come as outliers in PC 2 from stocks of another river.

Worth noting are the numerous activities currently taking place in the rivers joining L. Malawi which are heavily threatening *O. microlepis* population. Some of these detrimental activities are deliberate poisoning [22] and the deterioration of the spawning grounds in the rivers due to siltation from soil erosion caused by deforestation and agriculture [31]. In the long run, habitats and the whole riverine ecosystems have been deteriorated as water is abstracted from the breeding streams for irrigation and this makes it difficult for the juveniles to return to the lake from the spawning areas. Due to the scarcity of this species, the fish is currently hunted by numerous fishers as it fetches good prices [30]. The methods of capture have also shifted, as previously, the fish was caught using ring nets and by angling, but currently gillnets that are being used as active and passive gears are also being used in most of the rivers (such as Linthipe and North Rumphi), especially those rivers that have no rocks on their banks and beds. The gillnet is drifted down the river to the mouth, sometimes encircled around pools of water where the fish congregate during breeding, which gives no any chance for selectivity or fish to escape. The inadequacy of management measures in the major rivers (for example, close period, enforcement of mesh size restrictions and patrols) of Malaŵi has exacerbated the problems in the conservation of potamodromous fishes and other fish species residing in these rivers. In addition, recruitment overfishing and reduction of spawning stock below a critical threshold have prevented the species populations from rebuilding to previous levels of abundance in many rivers. Riverine potamodromous cyprinid species are most vulnerable because they are targeted by fishers during the spawning period when they swim upstream [22] for spawning. This development has led to the fishery of some tributaries of L. Malawi such as North Rukuru, Bua, Dwangwa and Linthipe to be threatened with extinction [40]. The whole scenario has posed a dire need for urgent attention to these rivers in order to save these potamodromous fish species as failure to do so risks complete harvest of the fish. Even though fisheries resources are renewable, the bad thing is that they are exhaustible and fisheries



resources are the most exploited resources of all the natural animal resources. Care must therefore be taken for this species in the rivers joining L. Malawi. Despite the change in the ecosystem, the interesting aspect is that the stock has not changed morphologically as has been unraveled in the current study, but reduced in number from the over-exploitation. The reduction in population of this fish species has been reported severally [11, 12, 14, 15, 17, 19, 22, 31, 32, 40] and the current study is evidencing the same. The fact that the species has not changed morphologically and that the stocks of the rivers joining the lake do not significantly differ, gives an opportunity that measures applicable for the reversal of the threat that *O. microlepis* is in can be universal and be utilized to all the rivers into which the species migrate for spawning and an immediate action in the conservation of the species can change the situation at hand. Results from a genetic study by Changadeya *et al.* [40] revealed that the *O. microlepis* from the rivers have high genetic variation as well as results for Chigamba *et al.* [41] which used life history traits found minor morphological differences. Results in the current study indicate no significant differences in morphological features and the authors recommend another study that combines an investigation of *O. microlepis* in the rivers adjoining L. Malawi using morphometrics, genetic diversity and structure, and life history studies at once to ascertain the results. Currently, the results of this study unravel the fact that the stocks of *O. microlepis* from the studied rivers are morphologically similar and application of the management measures in the rivers should be now and not later to save the *O. microlepis* in the rivers.

CONCLUSION, AND RECOMMENDATIONS FOR DEVELOPMENT

The study confirms the hypothesis that there are no significant differences in the morphological features of *O. microlepis* from the rivers Linthipe, Bua and North Rumpi signifying that the stocks of these rivers are morphologically similar despite migrating from L. Malawi to different rivers for breeding in their spawning season. The results in the current study portray that the fish in the studied rivers do not differ morphologically and that the populations can be managed as one since they are morphologically similar. These results deviate from the previous studies on genetic diversity and structure as well as life history traits where the populations were found to have high genetic variations. The study therefore concludes that management measures suggested in this paper that include regulations on mesh sizes, closing rivers from fishing during spawning period and all other measures applicable can be employed uniformly to manage the species in the major tributary rivers that connect to L. Malawi where the species migrate for breeding in order to save the species from completely getting extinct.



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DISCLAIMER

Findings, opinions, conclusions and recommendations expressed in this material are those of the author(s) and the funding agencies do not accept any liability in this regard.

CONFLICT OF INTEREST

The authors of this paper declare that there is no conflict of interest in relation to this work.

AUTHORS' CONTRIBUTIONS

Khumbanyiwa DD came up with the idea of the study, did the sampling, data analysis and drafting of the manuscript; Kaunda E, Singini W, Limuwa M and Jere W reviewed the idea as well as the manuscript and also supervised the whole study, as well as interpretation of data. All authors have commented and approved the final version of the manuscript.

APPROVAL FOR PUBLICATION

All co-authors have approved the publication of this work

DATA AVAILABILITY STATEMENT

Data generated in this study can be provided upon request from the corresponding author.



Table 1: Sampling of fish in each month and river (n = 111)

| Month | Number of fish | | | Days |
|--------|----------------|----------|--------------|------|
| | Bua | Linthipe | North Rumpfi | |
| March | 6 | 10 | | 3 |
| April | 9 | 10 | 4 | 4 |
| May | 15 | 10 | | 3 |
| June | 9 | 10 | | 3 |
| July | 10 | 6 | | 3 |
| August | 10 | 2 | | 4 |
| | 59 | 48 | 4 | |

Table 2: Morphometric characteristics used in measuring *O. microlepis* in the present study

| No | Characteristic | Abbreviation | No | Characteristic | Abbreviation |
|----|--------------------------------------|--------------|----|---------------------------------------|--------------|
| 1 | Standard length | SL | 13 | Posterior dorsal to pelvic-fin origin | PDP2 |
| 2 | Head length | HL | 14 | Caudal-peduncle length | CPL |
| 3 | Snout to dorsal-fin origin | SNDOR | 15 | Least caudal-peduncle depth | LCPD |
| 4 | Snout to pelvic-fin origin | SNPEL | 16 | Body length | BL |
| 5 | Dorsal-fin base length | DFBL | 17 | Snout length | SNL |
| 6 | Anterior dorsal to anterior anal | ADAA | 18 | Postorbital head length | POHL |
| 7 | Anterior dorsal to posterior anal | ADPA | 19 | Horizontal eye diameter | HED |
| 8 | Posterior dorsal to anterior anal | PDAA | 20 | Vertical eye diameter | VED |
| 9 | Posterior dorsal to posterior anal | PDPA | 21 | Preorbital length | PRE |
| 10 | Posterior dorsal to ventral caudal | PDVC | 22 | Cheek depth | CD |
| 11 | Posterior anal to dorsal caudal | PADC | 23 | Lower-jaw length | LJL |
| 12 | Anterior dorsal to pelvic-fin origin | APD2 | 24 | Head depth | HD |

Table 3: Kaiser-Meyer-Olkin (KMO) Test and Bartlett's test of sphericity results

| Kaiser-Meyer-Olkin | Measure of Sampling Adequacy | .966 |
|-------------------------------|------------------------------|----------|
| | Approx. Chi-Square | 5201.063 |
| Bartlett's Test of Sphericity | df | 276 |
| | Sig. | 0 |
| Anti-image Correlation | SL | .974 |
| | HL | .973 |
| | SNL | .934 |
| | POHL | .977 |
| | HED | .845 |
| | VED | .970 |
| | PRE | .964 |
| | CD | .982 |
| | LJL | .950 |
| | HD | .953 |
| | BD | .962 |
| | SNDOR | .967 |
| | SNPEL | .961 |
| | DFBL | .972 |
| | ADAA | .965 |
| | ADPA | .960 |
| | PDAA | .989 |
| | PDPA | .943 |
| | PDVC | .952 |
| | PADC | .968 |
| ADP2 | .966 | |
| PDP2 | .966 | |
| CPL | .986 | |
| LCPD | .986 | |

Table 4: Descriptive statistics of morphometric measurements

| Number | Characteristics | Bua | | | | Linthipe | | | | North Rumphu | | | |
|--------|-----------------|--------|--------|--------|----------|----------|--------|--------|----------|--------------|--------|--------|----------|
| | | Mean | Min | Max | Std. Dev | Mean | Min | Max | Std. Dev | Mean | Min | Max | Std. Dev |
| 1 | SL | 395.17 | 210.00 | 510.00 | 95.18 | 436.54 | 230.00 | 530.00 | 74.30 | 437.50 | 370.00 | 520.00 | 74.11 |
| 2 | HL | 96.83 | 50.50 | 151.30 | 23.97 | 105.87 | 58.80 | 126.15 | 12.97 | 102.19 | 87.20 | 119.35 | 16.67 |
| 3 | SNL | 32.56 | 15.50 | 46.70 | 8.64 | 37.40 | 17.60 | 135.15 | 15.32 | 31.96 | 26.40 | 37.55 | 6.14 |
| 4 | POHL | 51.04 | 25.85 | 69.10 | 13.03 | 55.91 | 29.10 | 68.80 | 7.59 | 53.63 | 42.90 | 65.20 | 10.27 |
| 5 | HED | 15.54 | 10.00 | 19.70 | 2.42 | 17.74 | 10.90 | 77.70 | 8.95 | 16.89 | 15.15 | 18.60 | 1.45 |
| 6 | VED | 14.17 | 8.80 | 17.85 | 2.31 | 15.01 | 9.55 | 18.60 | 1.37 | 15.84 | 14.80 | 16.80 | 0.89 |
| 7 | PRE | 29.25 | 13.45 | 42.00 | 7.95 | 32.64 | 16.05 | 45.85 | 5.50 | 30.04 | 25.00 | 35.85 | 5.75 |
| 8 | CD | 26.63 | 11.10 | 39.20 | 8.32 | 29.23 | 13.65 | 37.80 | 4.41 | 29.09 | 23.40 | 36.65 | 6.67 |
| 9 | LJL | 52.95 | 16.20 | 73.00 | 14.12 | 58.26 | 31.35 | 70.50 | 7.91 | 56.23 | 47.95 | 65.55 | 9.20 |
| 10 | HD | 55.28 | 16.70 | 78.90 | 16.37 | 61.05 | 29.65 | 77.15 | 8.27 | 61.96 | 53.80 | 72.80 | 9.45 |
| 11 | BD | 71.72 | 33.00 | 101.20 | 19.52 | 80.81 | 36.60 | 101.55 | 12.01 | 83.50 | 69.60 | 104.90 | 15.89 |
| 12 | SNDOR | 206.67 | 96.00 | 275.30 | 51.53 | 233.36 | 107.65 | 303.45 | 32.40 | 203.36 | 103.05 | 262.70 | 71.01 |
| 13 | SNPEL | 188.33 | 32.20 | 257.50 | 54.02 | 213.95 | 93.00 | 254.40 | 31.28 | 186.25 | 70.10 | 260.50 | 84.97 |
| 14 | DFBL | 46.67 | 16.30 | 67.40 | 12.08 | 52.27 | 24.90 | 68.30 | 7.86 | 50.94 | 43.55 | 58.20 | 8.02 |
| 15 | ADAA | 87.32 | 16.30 | 118.35 | 23.21 | 99.05 | 49.50 | 118.50 | 12.61 | 97.23 | 81.60 | 116.90 | 17.06 |
| 16 | ADPA | 110.07 | 31.90 | 224.75 | 33.53 | 125.05 | 61.30 | 222.65 | 20.67 | 124.56 | 106.25 | 143.00 | 19.95 |
| 17 | PDAA | 73.07 | 37.00 | 140.00 | 20.43 | 80.33 | 39.50 | 100.95 | 12.13 | 78.18 | 64.40 | 95.00 | 15.16 |
| 18 | PDPA | 69.75 | 37.30 | 98.20 | 16.76 | 78.13 | 37.20 | 90.90 | 9.31 | 77.34 | 62.50 | 96.70 | 14.82 |
| 19 | PDVC | 124.41 | 67.10 | 163.30 | 29.10 | 137.63 | 76.10 | 160.20 | 14.29 | 135.43 | 112.70 | 161.00 | 22.15 |
| 20 | PADC | 75.83 | 42.20 | 102.95 | 17.68 | 83.52 | 45.70 | 103.50 | 9.71 | 81.38 | 68.50 | 97.40 | 14.96 |
| 21 | ADP2 | 85.82 | 43.00 | 121.50 | 21.60 | 97.69 | 50.95 | 127.70 | 12.56 | 92.51 | 77.95 | 111.55 | 16.95 |
| 22 | PDP2 | 101.67 | 48.30 | 145.00 | 26.26 | 114.76 | 62.05 | 143.80 | 14.29 | 110.23 | 91.95 | 130.70 | 20.26 |
| 23 | CPL | 67.53 | 31.00 | 94.00 | 16.80 | 76.62 | 43.40 | 91.45 | 7.48 | 73.38 | 63.00 | 86.40 | 11.65 |
| 24 | LCPD | 33.02 | 16.30 | 45.15 | 7.92 | 36.90 | 19.60 | 44.90 | 4.51 | 36.53 | 30.15 | 42.00 | 6.18 |



Table 5: Results from Principal Component Analysis of *O. microlepis* species on the 24 morphological characteristics from three rivers (n = 111)

| | PCA axis | |
|---------------------------------------|-----------|--------------|
| | 1 | 2 |
| Eigenvalue | 19.60 | 1.02 |
| Percent variance | 81.67 | 4.24 |
| Standard length | 0.9216384 | 0.018108762 |
| Head length | 0.9739435 | -0.024924489 |
| Snout length | 0.6315680 | 0.205429515 |
| Posterior head length | 0.9686128 | -0.064571171 |
| Horizontal eye diameter | 0.2345158 | 0.914959486 |
| Vertical eye diameter | 0.8145275 | 0.268379181 |
| Preorbital length | 0.9361088 | -0.078101975 |
| Cheek depth | 0.9643529 | -0.004455941 |
| Lower jaw length | 0.9304340 | -0.055448614 |
| Head depth | 0.9365176 | -0.009361768 |
| Body depth | 0.9640258 | -0.054034136 |
| Snout to dorsal fin origin | 0.8986212 | 0.002825464 |
| Snout to pelvic fin origin | 0.8482417 | -0.004177366 |
| Dorsal fin base length | 0.9552584 | -0.042150814 |
| Anterior dorsal to anterior anal | 0.9677210 | -0.056073325 |
| Anterior dorsal to posterior anal | 0.8322822 | 0.151286384 |
| Posterior dorsal to anterior anal | 0.9011594 | -0.069214928 |
| Posterior dorsal to posterior anal | 0.9700954 | -0.030332454 |
| Posterior dorsal to vertical caudal | 0.9810640 | -0.027099523 |
| Posterior anal to dorsal caudal | 0.9758540 | -0.048361039 |
| Anterior dorsal to pelvic fin origin | 0.9631621 | -0.049276679 |
| Posterior dorsal to pelvic fin origin | 0.9603613 | -0.024173952 |
| Caudal peduncle length | 0.8671633 | -0.089300944 |
| Least caudal peduncle depth | 0.9663407 | -0.020298713 |

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