

Morphometric analysis of a Cyprinid species, *Enteromius paludinosus* (Peters, 1852), in the Lake Chilwa Basin, Malawi

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Abstract

Species divergence under geographically varying selection across disparate habitats has intrigued ecologists for decades. The Lake Chilwa inland drainage basin in Malawi has historically undergone wild water-level oscillations, sometimes being split into various sub-basins. Hydrological regime shifts may drive morphological variance in fishes. Although many scientists have studied fishes of this basin, we know little about the potential effects of its stochasticity on its ichthyofauna. Trait-based approaches are useful for detecting phenotypic changes among different populations. We used morphometric analysis to assess morphological disparities among three allopatric populations of *Enteromius paludinosus*, a fisheries-dominant cyprinid of this basin. Specimens from its three inlet rivers were measured on 18 traits and the data analysed using principal component analysis, the Welch-F test and t-tests in the Palaeontological Statistics package. Two river populations were clearly separated along seven traits: post-anal distance II, post-dorsal distance I, post-dorsal distance II, post-anal distance I, pre-anal distance, pre-pelvic distance and pre-dorsal. This divergence may be related to different abiotic selective pressures in their unique habitats, perhaps mediated by adaptive phenotype switching and incongruent growth trajectories. More eco-morphological studies in this basin may fully unravel the link between its wild hydrological fluctuations and the eco-evolutionary dynamics of its fishes.

Keywords: *Morphometric analysis, environmental fluctuation, Lake Chilwa, Enteromius paludinosus, divergence*

1.0 INTRODUCTION

Over the few past decades, there has been a lot of interest among ecologists in the divergence of species of various organisms into multiple habitat-specific differentiated groups (Powers, 1991; Hoffmann and Weeks, 2007; Koblmüller *et al.*, 2011). Among other influences, disparities in ecological factors across habitats may drive subtle geographically varying selection that leads to significant correlations between traits (morphological, physiological, biochemical or behavioural) and the environment and hence habitat-specific phenotypes (Endler, 1977; Mullen *et al.* 2009). Population genotypes may thus interact with heterogeneous habitats to result in environmentally induced deviations in phenotype, allowing for the existence of more than one alternative forms of morphology, behaviour or physiology in response to diverse environmental contexts (Hegrenes, 2001; Baker and Foster, 2002). Such plasticity may buffer populations against the effects of natural selection thereby maintaining genetic variation within a population, which may be the basis for trait divergence (Baker and Foster, 2002). In fishes, phenotypic plasticity in morphology, coupled with ecological opportunity have been vital in driving diversification (Danley and Kocher, 2001; Koblmüller *et al.*, 2011; Salzburger *et al.*, 2014; Rajvov *et al.* 2018). For instance, phenotypic plasticity in jaw morphology may drive variations in modes of feeding (Merona *et al.*, 2009).

One of the most ecologically important and fisheries-dominant cyprinid in Lake Chilwa, a shallow inland drainage basin in southern Malawi, is *Enteromius paludinosus* (Peters, 1852) (Jamu *et al.* 2003, Njaya *et al.*, 2011), an Afro-tropical small barb that is widely distributed in Africa (Skelton, 1993). The species occurs in marshes, small rivers and streams, as well as in the peripheral waters of larger lakes where it predominantly feeds on zooplankton, non-filamentous green algae and higher plant material (Bourn, 1974). A mostly diurnal feeder, younger *E. paludinosus* (less than 4.0 cm) tend to be more specialised in feeding largely on zooplankton while larger fish depict a more varied diet (Bourn, 1974). The species has a flexible life-history style, succeeding best in more stable environments but also capable of thriving in abiotically harsher environments where more specialized species fail (Jackson 1989).

Although the Lake Chilwa Basin is one of the most productive in Africa (Schuyt 2005, Delaney *et al.*, 2006), in some years contributing up to 30% of the total annual fish catches for Malawi (Schuijt and Jansen, 1999; Jamu *et al.*, 2011), the lake has a history of undergoing wild, mostly drought-induced, water-level fluctuations and has almost totally dried up twelve times between 1900 and 2015 (Garson, 1960; Moss and Moss, 1969; Morgan, 1972; Wilson, 2014; Nagoli and Chiwona-Karlton, 2017;

Kambombe *et al.*, 2021). Variations in water level are one of the critical environmental parameters that can deleteriously affect aquatic communities, including fish populations (Lancaster, 1979; Cantrell 1988; Benejam *et al.*, 2008; Nagrodski *et al.*, 2012; Nhiwatiwa *et al.*, 2017). Notwithstanding the fact that water levels of shallow lakes oscillate naturally across seasons and years as driven by climatic changes and anthropogenic activities (Blindow 1992, Coops *et al.*, 2003.), small inland lakes are particularly vulnerable to variations in water levels as such fluctuations have cascading influences over entire lake ecosystems (Evans *et al.*, 2005). For instance, under reduced water levels and hence attenuated water quantity and quality in shallow endorheic lakes, fish diversity dwindles due to lack of adequate conducive annual breeding environments (Jackson, 1989). During periods of desiccation, fishes of Lake Chilwa's Basin utilise its inflowing rivers and swamps as spawning grounds and refugia (Jamu and Brummett, 1999). Although numerous studies related to aspects of the distribution, migration and reproductive-breeding biology of the *Enteromius* and other fishes of the Lake Chilwa Basin have been conducted (Kirk 1967; Morgan 1972; Cantrell 1988; Jamu and Brummett, 1999; Jamu *et al.*, 2003; Delaney *et al.*, 2006; Macuiane *et al.*, 2009), very little, if any, work has been carried out to assess potential environmentally-driven ecomorphological divergence among the basin's fish populations. This is despite the fact that wild water level fluctuations and splitting of the aquatic environment around Lake Chilwa basin have occurred severally over time (Morgan, 1972; Wilson, 2014; Nagoli and Chiwona-Karltun, 2017; Kambombe *et al.*, 2021). Hydrological regime shifts can be drivers of fish morphologic divergence in shallow ephemeral lakes whose water levels may oscillate radically between remarkably high levels and complete drying (Talling, 1992, Dumont, 1992; Wantzen *et al.*, 2008; Kolding and van Zwieten, 2012; Bruckerhoff and Magoulick 2017, Peel *et al.*, 2019). Unravelling how phenotypic divergence in fishes may be underlain by environmental factors could provide a lot of insights into the evolutionary consequences of disrupting natural hydrologic patterns (Bruckerhoff and Magoulick, 2017) as the case is in the Lake Chilwa basin. The paucity of ecomorphological investigations on the fishes of the Lake Chilwa Basin is quite astounding, given the fisheries significance of this ichthyofauna in the country (Schuyt, 2005, Jamu *et al.*, 2011). Morphometric analyses have the potential to reveal incipient and oftentimes small phenotypic changes among different species populations (Kerschbaumer and Sturmbauer, 2011). Using morphometric analysis, this study assessed morphological disparities among three different allopatric *Enteromius paludinosus* populations in

the Lake Chilwa Basin in a bid to unravel potential phenotypic divergence among these fish populations.

2.0 MATERIALS AND METHODS

2.1 Data collection

Enteromius paludinosus (Peters, 1852) fish specimens were collected from Phalombe River at Miseu folo (15°40'25"S, 35°30'06"E)(n = 58), Sombani River at Phaloni (15°37'36"S, 35°45'22"E)(n = 52) and Likangala River at Lamusi (15°25'53"S, 35°32'30"E)(n = 79)(Figure 1). These three rivers are among the five most important in-flowing rivers of Lake Chilwa (Jamu *et al.* 2003; Macuiane *et al.* 2009). The sampling sites were chosen on the basis that they are locations of active artisanal fishing for this species and thus provided easy access to the samples from local fisher folks. The specimens were measured on 18 traits using a digital calliper with a precision of 0.01 mm according to procedures outlined in Snoeks (2004) and Bamba *et al.* (2011). Briefly, and in accordance with the definitions of these authorities (Snoeks, 2004; Bamba *et al.* 2011), the sizes of the following traits were determined: Standard length (SL), Head length (HL), Eye diameter (ED), Snout length (SnL), Interorbital width (IOW), Pre-dorsal distance (PrDD), Post-dorsal distance I (PoDD1), Post-dorsal distance II (PoDD2), Dorsal fin base length (DoFBL), Dorsal fin length (DoFL), Pre-pectoral distance (PrPecD), Pre-pelvic distance (PrPelD), Pre-anal distance (PrAD), Post-anal distance I (PoAD1), Post-anal distance II (PoAD2), Body depth I (BD1), Body depth II (BD2), Maximum caudal peduncle depth (MxCPD) and Minimum caudal peduncle depth (MnCPD). All measurements were expressed as ratios of a reference length; head measurements (Eye diameter, Snout length, Inter-orbital width) were expressed as percentages of head length while body measurements (Head length, Pre-dorsal distance, Post-dorsal distance I, Post-dorsal distance II, Dorsal fin base length, Dorsal fin length, Pre-pectoral distance, Pre-pelvic distance, Pre-anal distance, Post-anal distance I, Post-anal distance II, Body depth I, Body depth II, Maximum caudal peduncle depth and Minimum caudal peduncle depth) were recorded as percentages of standard length (Snoeks 2004; Bamba *et al.*, 2011).

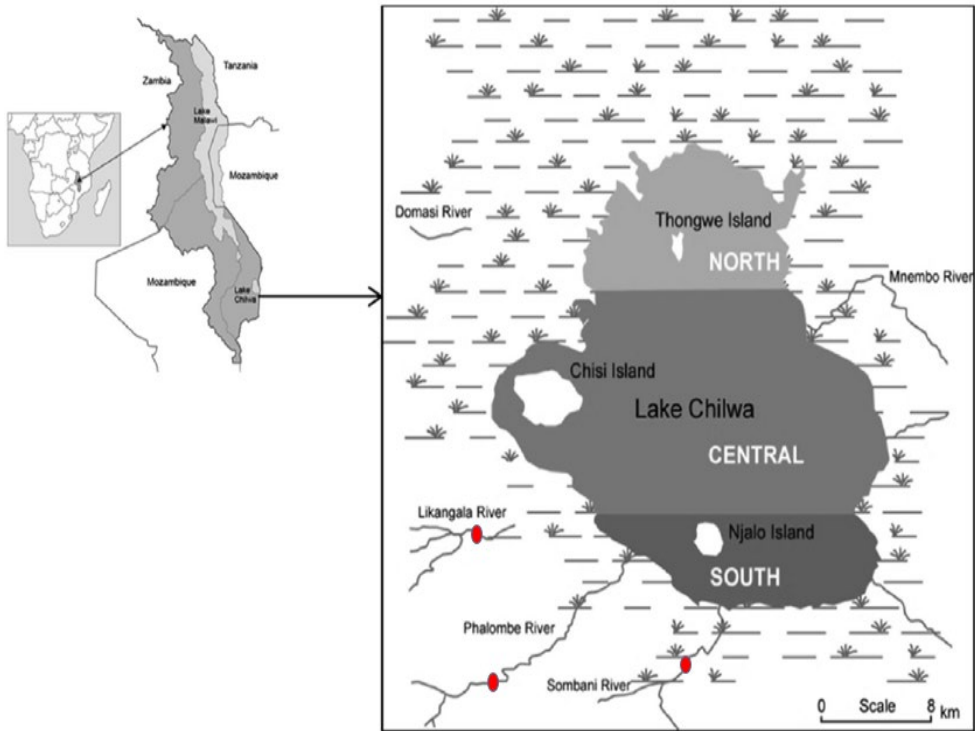


Figure 1: Map of the Lake Chilwa Basin in southern Malawi, showing the sampling points on Rivers Likangala, Phalombe and Sombani (red dots) for the *E. paludinosus* fishes that were used for morphometric analysis in this study. Adapted from Nagoli and Chiwona-Karltun (2017).

2.2 Data analysis

Since there was a significant difference in the size distributions (Standard length) of the three samples (Kruskal-Wallis test for equal medians, $H = 63.46$, $p < 0.0001$), which could mask the morphological differences between the three samples, the size component was removed from the shape measures by standardizing all individual morphometric trait measurements in accordance with the procedure of Elliot *et al.* (1995). Under this protocol, the potential effect of variation in size distributions (Standard length) of the concerned river samples on their shape measurements was counteracted by standardizing all individual morphometric character measurements using the formula:

$$M_s = M_o (L_s/L_o)^b$$

where M_s = Standardized measurement, M_o = Measured character length, L_s = Overall (arithmetic) mean standard length for all fish from all samples in each analysis, L_o = Standard length of the specimen. Parameter b is estimated for each character as the slope of the regression of $\log M_o$ on $\log L_o$ using all fish in all groups from the observed data by the allometric growth equation $M = aL^b$.

The measurements were then analysed using principal component analysis (PCA) to tease out differentiation of river-specific *E. paludinosus* groups on account of their morphometric traits and to pinpoint those traits that contributed most to the delineation of these cyprinid populations. Principle component analysis is a powerful tool for exploring a multivariate data matrix, reducing a large number of variables into a few biologically meaningful axes (Principal components) that are linear expression of the original variables (Snoeks, 2004). To further offset the effect of size, trait comparison analyses were performed only on samples belonging to the same length class. One way analysis of variance (ANOVA) and the Welch F test (in case of unequal variances) were used to compare trait means among the three populations. Statistical analyses were conducted in Microsoft Excel (2010) and Palaeontological Statistics (PAST) packages.

3.0 RESULTS

Principal component analysis revealed that the first four principal components accounted for about 95% of the morphological variation among the three *E. paludinosus* populations. The first principal component (PC1), normally a general size factor, accounted for about 88% of the morphological variation while the next two components, PC2 and PC3, together explained about 6% of the total morphological variance. A plot of the three populations of *E. paludinosus* (Likangala, Phalombe and Sombani Rivers) along PC2 and PC3 showed a clear separation between the Likangala and Phalombe populations along PC2 but no clear separation between the Sombani population and either of the two populations. There was no separation among the three populations along PC3 and no further analysis was conducted with respect to this component (Figure 2).

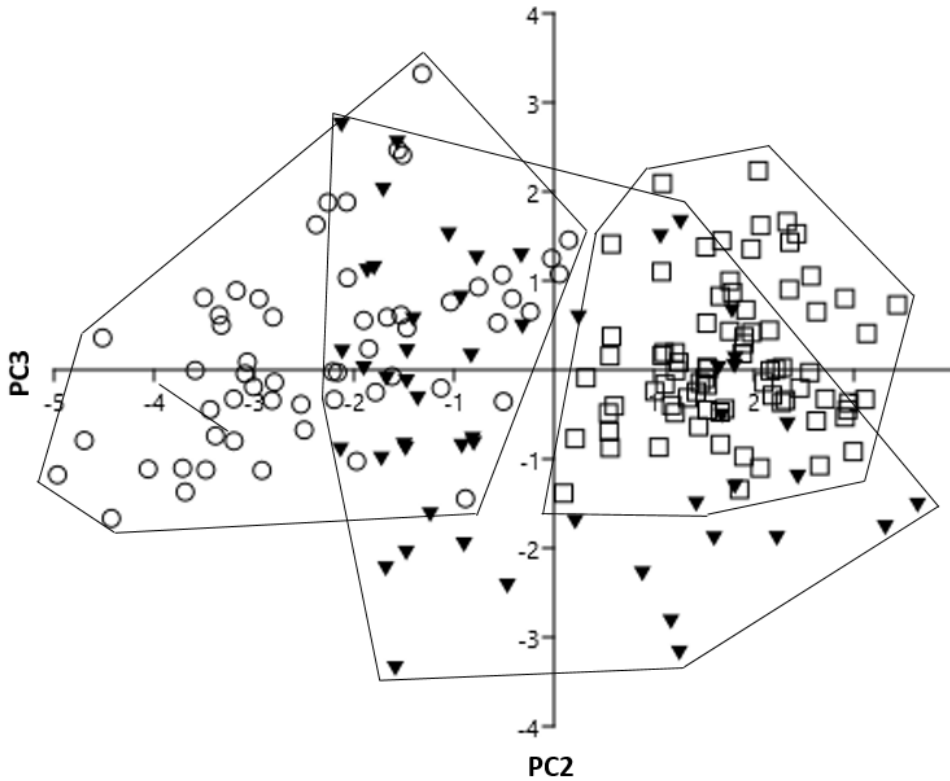


Figure 2: Principle component scatter plot (PC3 vs PC2) of three *E. paludinosus* populations from Likangala River (Unfilled squares), Phalombe River (Unfilled circles) and Sombani River (Inverted filled triangles), Lake Chilwa Basin, Malawi

Factor loadings for the first three principal components are shown in Table 1. All loadings on PC I were positive, an indication that this axis is a general size factor, largely a proxy for overall size. Post-anal distance I (PoAD1), Pre-anal distance (PrAD), Pre-pelvic distance (PrPelD), Post-anal distance II (PoAD2), Post-dorsal distance I (PoDD1), Post-dorsal distance II (PoDD2) and Pre-pectoral distance (PrPecD) dominated (had higher positive or negative values) the loadings on PC2 in that order. The Likangala and Phalombe *E. paludinosus* populations are thus delineated along these seven characters that loaded highly on PC2.

Table 1: Eigenvector loadings for principal component analysis of 18 external morphological measurements on three *E. paludinosus* populations from Likangala, Sombani and Phalombe Rivers, Lake Chilwa Basin, Malawi. Values of the first three principal components only are shown.

Character	PC 1	PC 2	PC 3
Standard length	0.7070	0.0123	-0.1421
Head length	0.0124	0.1531	-0.0063
Eye diameter	0.0302	0.1107	0.0727
Snout length	0.0272	0.0969	0.1022
Inter-orbital length	0.0773	0.1068	-0.0131
Pre-dorsal distance	0.2251	-0.1957	0.1264
Post-dorsal distance I	0.3086	0.3149	-0.4679
Post-dorsal distance II	0.2549	0.2696	-0.2961
Dorsal fin base length	0.0792	0.0356	-0.0581
Pre-pectoral distance	0.1134	-0.2505	0.0688
Pre-pelvic distance	0.2322	-0.4005	0.1983
Pre-anal distance	0.3394	-0.4266	0.1590
Post-anal distance I	0.1762	0.4269	0.6145
Post-anal distance II	0.1405	0.3594	0.4187
Body depth I	0.1185	-0.0048	0.0932
Body depth II	0.1326	-0.1055	0.0503
Maximum caudal peduncle depth	0.0812	0.0648	-0.0418
Minimum caudal peduncle depth	0.0655	0.0052	0.0509

Comparison of trait measurements between equal-sized groups of individuals ($t = -0.32$, $df = 131$, $p = 0.7529$) from Likangala River (50.36 ± 0.43 mm, range: 39.78 – 64.41mm, $n = 76$) and Phalombe River (50.12 ± 0.63 mm, range: 39.51 – 63.16 mm, $n = 57$) revealed that *E. paludinosus* from Likangala River had larger post-anal distance II, post-dorsal distance I and post-dorsal distance II but smaller post-anal distance I, pre-anal distance, pre-pelvic distance and pre-dorsal distance than *E. paludinosus* from Phalombe River (Table 2).

Table 2: Differences in some *E. paludinosus* morphological traits (mean mm \pm SE) between the Likangala and Phalombe River populations, Lake Chilwa Basin, Malawi

Measured trait	Likangala (n = 76)	Phalombe (n = 57)	t-value	df	p-value
Post-anal distance I	10.99 \pm 0.22	12.70 \pm 0.13	-6.46	96	<0.0001
Pre-anal distance	24.01 \pm 0.21	25.66 \pm 0.34	4.14	96	< 0.0001
Pre-pelvic distance	16.31 \pm 0.17	18.21 \pm 0.23	6.39	108	<0.0001
Post-anal distance II	9.39 \pm 0.11	7.99 \pm 0.19	-6.43	91	<0.0001
Post-dorsal distance I	21.09 \pm 0.20	19.67 \pm 0.31	-3.86	98	<0.0001
Post-dorsal distance II	15.74 \pm 0.18	14.69 \pm 0.26	-3.50	104	0.0006
Pre-pectoral distance	8.60 \pm 0.09	9.58 \pm 0.13	6.13	101	< 0.0001

Subsequent regression analysis was carried out on those traits in Table 2 against standard length to confirm if such disparity is due to different growth trends, following Kassam *et al.* (2002). The scatterplots of the seven traits (Figure 3) indicate that these populations differed significantly in their slope of post-anal distance II on SL (ANCOVA, $F = 7.82$, $p = 0.0060$)(Graph A) and their slope of post-anal distance I on SL (ANCOVA, $F = 9.37$, $p = 0.0027$) (Graph D), showing dissimilar growth rates between the two populations in the post anal area (higher in the Likangala River population than in the Phalombe River population). There were no significant differences (between the two populations) in the slopes of the rest of the other traits (Graphs B, C, E, F and G) on SL (ANCOVA, $p > 0.05$).

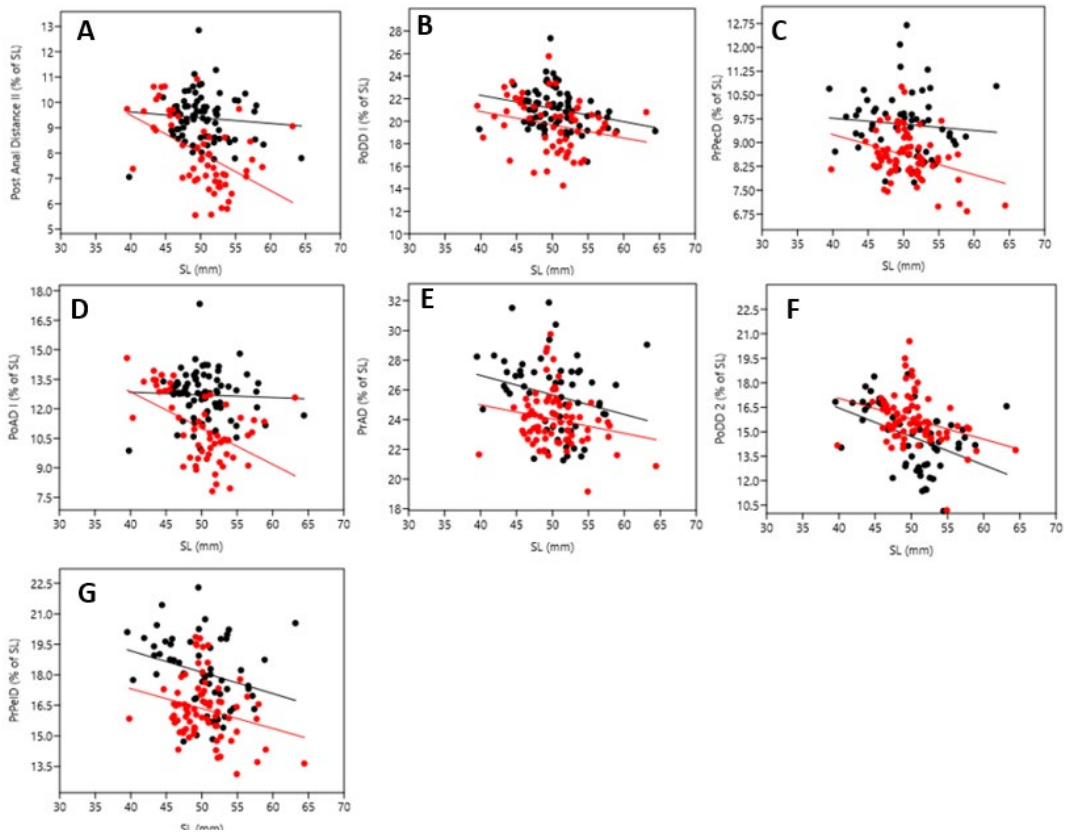


Figure 3: Bivariate plots showing relationships of the seven characters (A to G) versus standard length in depiction of growth trends of two *E. paludinosus* populations from Lake Chilwa Basin, Malawi. Black circles: Likangala population; red circles, Phalombe population; black circles. Only characters that loaded highly on PC II are presented

An analysis of variability in the measured traits (that differed between Likangala and Phalombe populations) based on the Fligner-Killeen test for equal coefficients of variation found that the Phalombe population had higher variance in the PoDD1 ($p = 0.0200$), PoDD2 ($p = 0.0287$), PoADI ($p = 0.0023$) and PoADII ($p = 0.0001$) than the Likangala population. No difference was found between the two river populations in the variability of PrPecD ($p = 0.5088$), PrPelD ($p = 0.6249$) and PrAD ($p = 0.1142$).

4.0 DISCUSSION

Morphometric analyses can be useful for quantifying, visualising, and comparing shape changes in studies dealing with, *inter alia*, static, ontogenetic or evolutionary allometry, adaptations and phenotypic plasticity (Kaliontzopoulou, 2011; Ili' *et al.*,

2019). This study, the first morphometric analysis of its kind in the Lake Chilwa Basin, has revealed subtle morphological disparities between two cyprinid *Enteromius paludinosus* populations inhabiting different rivers within the same drainage system, mostly in the post anal region (distance from the base of first anal-fin ray to middle of the caudal peduncle base and the distance from the base of last anal-fin ray to middle of caudal peduncle base) and post-dorsal region (Distance from articulation of first dorsal-fin ray to middle of caudal peduncle base and distance from articulation of last dorsal-fin ray to middle of caudal peduncle base). A variety of biotic and abiotic environmental factors like the presence of predators, the diversity of available prey items, available vegetation cover and water velocity may trigger divergence of the head and body morphology in fishes (Bronmark and Miner, 1992, Pakkasmaa and Piironen, 2001, Olsson and Eklov, 2005; Heermann *et al.*, 2007, Vehanen and Huusko, 2011). Within-species morphological differences thus prevail in many fish groups that occupy environments with divergent abiotic or biotic selective pressures (Foster *et al.*, 2015; Bower *et al.*, 2018).

Post-anal shape variations in fish may indicate differences in elongation rates in this posterior part of the body consistent with divergent adaptive evolution favouring anguilliform locomotion in high-energy aquatic environments (Polgar *et al.*, 2017), with fishes in fast-flowing waters tending to have longer caudal regions and more streamlined body shapes associated with increased manoeuvrability, propulsion and resilience for long-distance migrations (Pakkasmaa and Piironen, 2001, Dasilao *et al.* 2002, Fraser *et al.* 2007). For instance, in *Morone saxatilis* and *M. chrysops* fishes, post-anal variations are among the primary delineating features among its populations, distinguishing between two adaptive swimming abilities, namely ‘cruisers’ or ‘accelerators’, such differences being correlated with variances in foraging behaviour (Webb and Weihs 1986; Fulford and Allen Rutherford, 2000). The morphological differences detected between these Cyprinid *Enteromius* fishes in this lake basin may suggest that different environmental conditions unique to their rivers underpin the asymmetrical growth trajectories of their body morphologies. Among other influences, disparities in ecological factors across habitats have been known to drive subtle geographically varying selection that may result in habitat-specific phenotypes (Endler, 1977; Kussell and Leibler 2005; Mullen *et al.*, 2009). Phenotypic plasticity, an evolutionary strategy by which an organism can alter the expression of its genotype including its morphology or behaviour may also be critical for niche specialization, a key innovation for evolutionary change (Baker and Foster, 2002). Fishes are well known for such phenotypic plasticity in their traits in relation to environmental conditions, which allows them to optimise their fitness in each environment (Mérona *et al.*, 2009). For instance, much greater phenotypic variability abounds for body size and growth rate patterns in fish than other vertebrates (Kerschbaumer and Sturmbauer, 2011). Phenotypic differences may result from stochastic micro-environmental variations that heterogeneously impact

on development trajectories (Simons and Johnston 1997; Bruijning *et al.*, 2019). Our results attest to the fact that the interaction between populations and diverse habitat contexts may result in environmentally induced deviations in phenotype, allowing for the existence of more than one alternative forms of morphology, behaviour or physiology (Hegrenes, 2001; Bouton *et al.*, 2002). Species that survive periodical dry-out phases of shallow tropical lakes as relict communities in disjointed nearby deep pools of perennial streams (main source of ‘inocula’ from which the refilling lakes get recolonized) are normally physiologically hardy and phenotypically plastic (Jackson 1989). The subject of this study, *E. paludinosus*, is known to have a flexible life-history, thriving best in unstable environments but also able to increase very greatly in numbers in harsher abiotic environments where more specialized species would otherwise perish (Jackson 1989). The nature of a habitat’s hydrological environment is a key factor in moulding and maintaining an individual fish’s morphology through a phenotypic plastic response (Dunn *et al.*, 2020). Through phenotypic plasticity, body morphology can respond to selective forces in the immediate term while responding through adaptation over longer timescales (Husemann *et al.*, 2017). The results of this study point to the possibility that these *E. paludinosus* populations have the propensity for morphological phenotypic plasticity, perhaps spurred by their interaction with the complex set of heterogeneous habitats they are exposed to during low water levels, which is a phenomenon the fishes have experienced in this lake since early 1900s.

Water levels of the Lake Chilwa Basin can fluctuate wildly and the lake itself has almost totally dried up more than ten times between 1900 and 2015 (Garson, 1960; Morgan, 1972; Moss and Moss, 1969; Njaya *et al.*, 2011; Wilson, 2014; Nagoli and Chiwona-Karlton, 2017, Kambombe *et al.* 2021). The physico-chemical nature of its waters also varies seasonally, mainly driven by geological history of fall in lake level, dilution with rainfall and river inflow from the catchment area, seasonal changes in the surface area of the lake, chemical interaction of some ions with each other and its exposed mud, concentration with evaporation and by biological utilisation of its ions (Morgan and Kalk, 1970, Jamu *et al.* 2003). Environmental fluctuations and resultant habitat quality variations may be a significant driving force behind changes in species composition and diversity and may contribute to morphological divergence among populations (Moss and Moss, 1969; Lande, 1993; Petchey, 2000; Acar *et al.*, 2008; Vehanen and Huusko, 2011). In this water basin, for instance, mass mortalities of *Tilapia* fishes have coincidentally occurred with peak levels in water conductivity in concert with high winds and resultant sediment re-suspension, increased turbidity and temporary dips in dissolved oxygen (Morgan and Kalk, 1970). Wild seasonal variations in salt concentrations have also been associated with apparent stunting of *Tilapia* fish species (Morgan and Kalk, 1970) as well as coincidental successional shifts in the major groups of zooplankton

(Cladocerans and copepods) and algae (green algae and blue-green) (Moss and Moss, 1969; Morgan and Kalk, 1970, Kalk 1979).

Although water level fluctuations affect all aquatic biota, fish are one of the most disproportionately impacted taxa through enhanced wind-driven sediment re-suspension and asphyxiation, altered behaviour, suffocation of eggs, reduction of available critical habitats and limited natural dispersion of their larvae and juveniles (Benech *et al.* 1983, Lande, 1993; Coops *et al.*, 2003). The disappearance of water flow can also affect fish's general growth (Coops *et al.*, 2003). The disjointedness of the aquatic habitats of the Lake Chilwa Basin during its periodic draw-down phases could be throwing *E. paludinosus* populations into separate discreet ecological contexts with dissimilar environmental variables capable of heterogeneously affecting the growth trajectories of these fishes.

Variation is a critical component that defines the adaptive capacity of a population; increased trait variation can drive substantial eco-evolutionary changes (Lande 2009, Forsman 2014; Nonaka *et al.*, 2015, Salinas *et al.*, 2019) and populations that possess sufficient phenotypic variation are likely to adapt to changing environments (Lande 1976, Bruijning *et al.*, 2020). An analysis of variability in the measured traits showed that the Phalombe population had higher variance than the Likangala population in three of the traits that delineated these two populations. Unique or rare environmental conditions may occasionally facilitate the unearthing of hitherto unseen phenotypes, simultaneously and ultimately leading to an upsurge in trait variation (Ghalambor *et al.*, 2007, Salinas *et al.*, 2019). We postulate that the population in Phalombe may have been exposed to more or harsher habitat-specific selective environmental forces than the Likangala one, presenting a skewed ecological opportunity for more trait divergence. The Sombani River population overlapped with that of the other two rivers and showed no separation from them. Although empirical phylogenetic evidence is required, it may be the case that this Sombani population is the main primary parental restocking source for Lake Chilwa when it recovers from its occasional dried-up state and that the other two rivers are restocked from this lake 'inoculum'.

In ecological studies, trait-based approaches for describing communities and their propensity for responding to natural or anthropogenic-driven environmental changes are becoming increasingly useful (Caillon *et al.*, 2018). As an integrative trait, morphology is ecologically useful in combining both functional and evolutionary information (Caillon *et al.*, 2018). This study is one of the very first to attempt a morphometric analysis of *E. paludinosus* species in this heavily fluctuating water basin and its findings attest to the fact that these types of analyses are a powerful tool not for only describing organismal shape variation but also detecting incipient and oftentimes small phenotypic changes among different populations of a species

(Ili' *et al.*, 2019, Arendt 2010, Kerschbaumer and Sturmbauer, 2011). In elucidating morphological disparities among species populations and pinpointing the actual traits that account most for species divergence, morphometric analysis may yield results that are in impressive alignment with those obtained through modern molecular techniques (Lovrenčić *et al.*, 2020). It is imperative that more morphometric studies coupled with molecular based analyses be undertaken in this water basin to unravel the influence of its environmental hydrological stochasticity on the eco-evolutionary dynamics of its fishes.

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