Notes and Records

Small-scale morphological differentiation in a cichlid may provide clues about rapidly diversifying systems

Rebecca Jordan[1]*, David Howe[1], Karen Kellogg[2], Francis Juanes[3], Jay Stauffer Jr[4] and Eva Garcia-Vazquez[5]

[1]*Ecology, Evolution, and Natural Resources, Cook College, Rutgers University, New Brunswick, NJ 08901, U.S.A.,* [2]*Environmental Studies, Skidmore College, Saratoga Springs, NY 12866, U.S.A.,* [3]*Natural Resources Conservation, University of Massachusetts, Amherst, MA 01003, U.S.A.,* [4]*Forest Resources, The Pennsylvania State University, University Park, PA 16802, U.S.A. and* [5]*Biologia Funcional, Universidad de Oviedo, C/Julian Claveria s/n, 33006 Oviedo, Spain*

Introduction

The diversification of Lake Malaŵi cichlid fishes has been characterized as the fastest speciation event within a single vertebrate family. In an effort to understand the population isolation mechanisms that contribute to the diversification of these cichlids, many researchers are studying population variation (Markert, Danley & Arnegard, 2001) along horizontal (i.e. constant depth) gradients which often include disrupted habitats. Little work, however, has been devoted to investigating population isolation along vertical scales. The vertical cline, in opposition to the horizontal, necessarily introduces gradients in both water pressure and the spectral quality of light (Loew & Mcfarland, 1990). These cichlids, which are able to travel across depths (Hill & Ribbink, 1978), rely heavily on vision for feeding and mate selection (e.g. Jordan et al., 2003). Changes in light may affect behavioural and physiological changes in both feeding and mate selection. Given the predominance of bimodal population distributions found along depth gradients in Lake Malaŵi (Ribbink et al., 1983), investigation into population variation along a vertical axis is warranted.

In this paper, we discuss results of an analysis of morphological variation in Metriaclima melabranchion, a philopatric cichlid found along the rocky shore of Lake Malaŵi. Tsano Rock, in southern Lake Malaŵi (14°S and 35°E), houses a bimodal distribution of M. melabranchion (Ribbink et al., 1983) with peaks in population size separated by 20 m in depth along an estimated 40-m continuous stretch of rocky habitat. Males of the species are light blue with black barring. We quantified the light environment at these locations and tested the null hypothesis that two population subsets, one taken from 3 and one from 23 m, are morphologically similar.

Methods

In January 1999, we collected ten fish from each depth (3 m and 23 m) that were immediately preserved in 10% buffered formalin. Following Stauffer & Hert (1992), external counts and measurements except for gill rakers were made on the left side of the twenty preserved fish (ten from 3 m and ten from 23 m). We analysed differences in body shape with a sheared principal components analysis (SPCA) of the morphometric data (Humphries et al., 1981) using the covariance matrix. We excluded pelvic or pectoral fin lengths because these measurements depend on reproductive stage (Stauer & Hert, 1992). We used principal components analysis (PCA) to analyse the meristic (i.e. count) data using the correlation matrix.

To inspect for differences between the groups, we plotted either the sheared second or third principal component of the morphometric data against the first principal component of the meristic data (i.e. factor score; Stauffer & Hert, 1992). We then used multivariate analysis of variance (MANOVA) in conjunction with a Hotelling–Lawley trace to determine whether the mean multivariate scores of the clusters formed by the minimum polygons of the PCA scores differed significantly (i.e. $P < 0.05$). Finally, as a measure of the robustness of the dataset, we randomized the sheared PCA dataset and repeated the analysis five times.

*Correspondence:* E-mail: jordan@aesop.rutgers.edu

The SPCA indicated that vertical and horizontal eye diameter, cheek depth and distances between the posterior insertions of the dorsal and anal fins and between the posterior insertion of the dorsal fin to the dorsal tip of the caudal fin loaded most heavily. Given that eye diameter and cheek depth loaded particularly high, we conducted a set of *post hoc* analyses. First, using analyses of covariance (ANCOVA), we investigated eye diameter by group (i.e. shallow or deep) and controlling for standard length (model: vertical/horizontal eye diameter = length + group + group × length + b). Length was measured from snout to mid-origin of the caudal fin. The interaction between group and length was not significant and was therefore removed from the model. Next, we investigated the relationship between cheek depth and eye diameter using regression analysis.

To assess how light, and in particular colour, changes with depth, photographs of spectrum paper (400–700 nm; RIT, Rochester, NY, U.S.A.) were taken at the surface (white light) and approximately every 6 m from the surface down to 23 m with an underwater digital camera insensitive to ultraviolet and infrared light. These pictures were downloaded into a graphic software program and intensity was measured along a wavelength transect run across the image. All data were referenced to white-light image values.

Results

We noted differences in the light environment between 3 and 23 m (Fig. 1). We also found significant morphological differences in shape (MANOVA, 19; $P < 0.001$), but not in counts (MANOVA, 19; $P = 0.088$) (Fig. 2). In addition, we found significant group separation by depth (Hotelling–Lawley, 17; $P < 0.001$). We found no significant differences (i.e. MANOVA, 18; $P > 0.050$, for all five randomized trials) among data clusters in the randomized data sets lending support to the robustness of our sample.

Controlling for body size, we found that eye diameter (both horizontally and vertically) was significantly smaller in the fish that inhabited the deeper depth (ANCOVA, 17; $P < 0.001$ for both analyses). We found a positive relationship between vertical and horizontal eye diameter and cheek depth [regression, 19; $P = 0.006$ (horizontal eye diameter), $P = 0.003$ (vertical eye diameter), with R^2 values of 34.5 and 40 respectively].
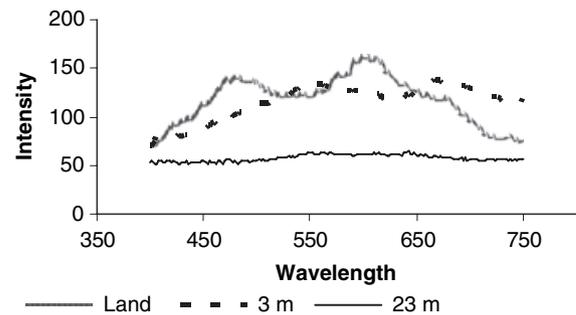
Fig 1 Intensity measured from a transect across images of spectrum paper. Each line represents data from images taken of the spectrum paper on land and at both depths. On land, intensity peaks at 600 nm with a slightly lesser peak at 480 nm. Two peaks were also seen at 3-m depth, although less intense, more similar to each other, and shifted upward in wavelength to 550 and 680 nm. At 23 m, however, much colour information is lost, intensity is decreased by 43%, and peaks are much reduced as compared with the shallow depth
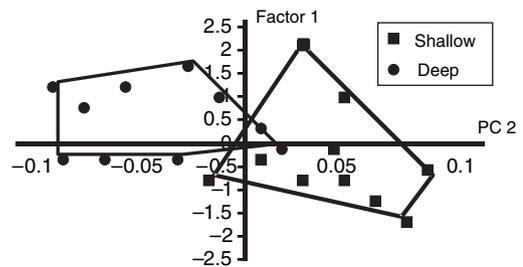
Fig 2 Below is a graph plotting the first factor score (meristic data) against the second principal component (morphometric data). This plot shows the separation between the two population subsets. This separation is significant along principal component (PC) 2 but not along factor 1 (MANOVA, 19; $P < 0.001$ and $P = 0.088$ respectively).

Discussion

We found significant morphological separation, characterized mostly by eye diameter differences, between two population subsets of *M. melabranchion* separated by 20-m depth. The reduced eye diameter with depth might suggest adaptation to the light environment. While it may seem intuitive that organisms in restricted light environments increase their eye size to capture more light (Kröger & Fernald, 1994), the fish from the greater depth may have smaller eyes in correspondence to the reduced transmission. Bouton, De Visser & Barel (2002) found a positive relationship between eye size and transmission spectrum

width in some Lake Victoria cichlids. The visual information available would differ between the two depths. For example, as inferred from the spectrum photographs, the lighter blue body areas of *M. melabranchion* might be slightly visible at the greater depth, but the overall contrast would be much reduced compared with fish in shallower water.

In addition to eye diameter, cheek depth was smaller at the deeper location. It is possible that jaw adaptation to different food types could translate into cheek depth differences, especially considering the phenotypic plasticity demonstrated in cichlid species (Witte, Barel & Hougerhoud, 1990). Given the implications of our results in terms of both scale and added dimensionality to the ecology behind potential population isolation, as well as the prevalence of bimodal distributions along depth gradients in Lake Malawi cichlids (Ribbink *et al.*, 1983), we suggest that the further study of genetic and morphological structure along vertical axes will provide novel insights into the study of species divergence.

Acknowledgements

References

BOUTON, N., DE VISSER, J. & BAREL, C.D.N. (2002) Correlating head shape with ecological variables in rock-dwelling haplochromines (Teleostei: Cichlidae) from Lake Victoria. *Biol. J. Linnean Soc.* **76**, 41–48.

HILL, B.J. & RIBBINK, A.J. (1978) Depth equilibrium of a shallow-water cichlid fish. *J. Fish Biol.* **13**, 741–745.

HUMPHRIES, J., BOOKSTEIN, F., CHERNOFF, B., SMITH, G., ELDER, R. & POSS, S. (1981) Multivariate discrimination by shape in relation to size. *Sys. Zool.* **30**, 291–308.

JORDAN, R.C., KELLOGG, K.A., JUANES, F. & STAUFFER, J.R., Jr (2003) An evaluation of female mate choice cues in a group of Lake Malaŵi Cichlids. *Copeia* **2003**, 181–186.

KRÖGER, R.H. & FERNALD, R.D. (1994) Regulation of eye growth in the African cichlid fish *Haplochromis burtoni*. *Vis. Res.* **34**, 1807–1814.

LOEW, E.R. & MCFARLAND, W.N. (1990) The underwater visual environment. In: The Visual System of Fish, 1st edn (Eds R. H. DOUGLAS and M. B. DJAMGOZ). Chapman and Hall, New York.

MARKERT, J.A., DANLEY, P.D. & ARNEGARD, M.E. (2001) New markers for new species: microsatellite loci and the East African cichlids. *Trends Ecol. Evol.* **16**, 100–107.

RIBBINK, A.J., MARSH, B.A., MARSH, A.C., RIBBINK, A.C. & SHARP, B.J. (1983) A preliminary survey of the cichlid fishes of rocky habitats in Lake Malaŵi. *S. Afr. J. Zool.* **18**, 149–310.

STAUFFER, J.R., Jr & HERT E. (1992) *Pseudotropheus callainos*, a new species of *mbuna* (Cichlidae), with analysis of changes associated with two intra-lacustrine transplantations in Lake Malaŵi, Africa. *Ichthyol. Explor. Freshw.* **3**, 253–264.

WITTE, F., BAREL, C.D.N. & HOUGERHOUD R.J.C. (1990) Phenotypic plasticity of anatomical structures and its ecomorphological significance. *Neth. J. Zool.* **40**, 278–298.