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# FINAL RPORT

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## Genetic Analysis of Stock Identification of Commercially Important Fishes from Malawi

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## Executive Summary

Approximately 80 species have been collected representing 29 genera. Sixteen new species have been described (see attached list of publications) and a revision of the *Pseudotropheus zebra* complex will be completed within a year based on collections made as part of this project. The use of behavioral characteristics in species descriptions was utilized for the sand-dwelling cichlids. A fish musuem for the University of Malaŵi was created and a laboratory for conducting starch gel electrophoresis was established at the University of Malaŵi.

## RESEARCH OBJECTIVES

The rift valley lakes of Africa harbor the most diverse ichthyofauna of any of the world's lakes. The shallow water endemic haplochromine fishes can be divided into two ecological groups -- the rock dwelling cichlids (*mbuna*) and the sand-dwelling cichlids. Within the *mbuna* a generic revision is sorely needed and many species are still undescribed; however, because of their popularity among aquarists and their distinctive color patterns, many species groups are phenetically recognized and numerous taxa have common names. The status of many of the sand-dwelling cichlid groups remains unresolved. The sand-dwelling taxa are not as dramatically colored and the distribution of many of the taxa is not known. In fact, type localities are not given for a large portion of the original descriptions. Of the type localities that can be found in the literature, nearly half are recorded simply as Lake Nyasa. The sand-dwelling fishes endemic to Lake Malaŵi are responsible for providing some 70% of the animal protein consumed by Malaŵians. In order to implement an effective plan to manage this fauna for maximal sustainable yield, the species must be identified. At a bare minimum, we need to collect morphological information from the type series, collect topotypes for genetic and morphological data, collect behavioral data from topotypes, and attempt to match the type series with extant populations in Lake Malaŵi. Our specific goals were to collect specimens of the commercially important species in southern Lake Malaŵi, measure breeding bowers of the shallow lek breeding species, use electrophoretic techniques to examine genetic similarity of target groups, and identify species by analyzing both morphometric and behavioral (as expressed by bower shape) data.

## METHODS AND RESULTS

**Sheared Principal Component Analysis (PCA).** Morphology has always played an important role in the study of the systematics and evolution of organisms. As part of these studies, attempts have been made to qualify and quantify the shapes of organisms. In the past, biological shapes have been delineated by a single measurement or a small number of measurements (Oxnard 1978) which have been standardized by the use of ratios (Strauss 1980). The use of ratios is now generally believed to be statistically invalid when delineating among groups (Humphries et al. 1981, Atchley 1978, Bookstein et al. 1985, Reyment et al. 1984, Mosimann and James 1979). Historically, the first principal component has been regarded as a size component; the additional components are considered to be dependent on the shape of the individual. The validity of this technique has also been questioned because there is a residual effect of size on components other than the first one. Consequently, a sheared principal component analysis was developed by Humphries et al. (1981) that restricts the variation due to size to the first component, so that subsequent components are strictly shape related. Stauffer and Boltz (1989) utilized this technique successfully to distinguish between two sympatric species of rock-dwelling fishes from Lake Malaŵi, and Stauffer (1991) used this technique to delimit populations of *Pseudotropheus pursus* from *Pseudotropheus livingstonii* and *Pseudotropheus lanisticola.*

Approximately 80 species have been collected representing the following genera: *Alticorpus, Auloncara, Buccochromis, Chilotilapia, Copadichromis, Cynotilapia, Cyrtocara, Dimidiochromis, Diplotaxodon, Genyochromis, Hemitilapia, Iodotropheus, Labeotropheus, Lethrinops, Mylochromis, Melanochromis, Nimbochromis, Oreochromis, Otopharynx, Petrotilapia, Protomelas, Pseudotropheus, Rhamphochromis, Serranochromis, Stigmatichromis, Taeniolethrinops, Tramitichromis, Trematocranus,* and *Tyrannochromis.* Color notes and

photographs were taken for the majority of fishes, which have been collected. Specimens were anesthetized and preserved in 10% formalin so that all fins were erect and the body straight.

A total of 33 counts and measurements of some 1000 specimens of the *Pseudotropheus zebra* complex have been completed. It is estimated that a monograph on this species complex will be completed by October, 1995.

A paper describing hybrids between *P. zebra* and *Cynotilapia afra* (Stauffer et al. in press) will be the first description of *in situ* hybrid fishes from Lake Malawi.

We used these techniques to analyze data from the type specimens of fishes that were provisionally classified as *Copadichromis eucinostomus*. When the sheared second principal component (morphometric data) is plotted against the first factor score (meristic data), there is no overlap between the clusters formed by three new species (*Copadichromis conophorus*, *Copadichromis cyclicos*, and *Copadichromis thinos*) and previously described fishes in what we regard as the *C. eucinostomus* species group (Stauffer et al. 1993: 1025, Fig. 10). Size accounts for 92% of the total variance, and the second principal component accounts for 2.8%. Those variables, which have the highest loadings on the sheared second principal component are vertical eye diameter, preorbital depth, distance between the posterior dorsal fin and the posterior anal fin, horizontal eye diameter, and caudal-peduncle length. The first principal component of the meristic data explains 53.1% of the variance. The variables, which have the highest loadings on the first factor score are the number of gill rakers on the ceratobranchial (0.30), dorsal-fin rays (0.27), and number of cheek scales (-0.28). *Copadachromis prostoma* differs from all other members of this group in both sheared second principal components (morphometric data) and the first principal components of the meristic data (Stauffer et al. 1993:1025, Fig. 10). It is further distinguished because of the presence of a dark lateral stripe. Similarly, *C. flavimanus* and *C.*

*inornatus* are quite distinct from the three species described and are separated primarily along the factor score. One could argue that shape differences may be in part related to the comparison of old and freshly preserved material; however, it is doubtful that meristic characters of preserved specimens would change with time.

In an attempt to further resolve the differences among the three species described herein and *C. eucinostomus*, with which they were previously confused, we examined the data for these four species independently of other members of the complex. There is no overlap between the clusters formed by *C. conophorus*, *C. cyclicos*, and *C. thinos* and that formed by *C. eucinostomus* (Stauffer et al. 1993:1025, Fig. 10). Additionally, there is no overlap between *C. conophorus* and *C. cyclicos*.

Observations have demonstrated that different genera can be distinguished by bower shape (McKaye, 1991). Moreover, preliminary evidence has indicated that members of different genera exhibit different courtship dances. For example, *Copadichromis* species have a circular courtship movement, as contrasted to sympatric *Tramitichromis* spp., which use a figure-eight courtship movement (Stauffer et al. 1993). Among the three new species described by Stauffer et al. (1993), no differences among courtship patterns of breeding males could be discerned; however, since the spawning platforms of each species are constructed by breeding males, we interpreted bower shape as a manifestation of a behavioral characteristic and used this character to help to delimit the species. The bowers constructed by *C. thinos* were generally smaller than those built by the other two species, and they were found in deeper water. A plot of the sheared second principal component against the sheared third principal component demonstrated that the bower shapes of the three species were different (Stauffer et al. 1993:1025, Fig 11). There was no overlap between the clusters formed by *C. conophorus* and *C. cyclicos*. The bowers of *C. thinos*

were intermediate in shape between the above two species; however, all three groups were significantly different ($P<0.05$) from each other, along the sheared second principal component axis (ANOVA, Duncan's multiple range test).

The same technique was applied to the type material of the described species of *Tramitichromis*. When the sheared second principal component (morphometric data) was plotted against the first principal component of the meristic data, the data from the syntypes of *Tramitichromis variabilis* from Jua (northern Lake Malaŵi) were completely disjunct from the cluster formed by syntypes of *T. variabilis* from southern Lake Malaŵi. Moreover, neither of these populations overlap with the lectotypes, which were collected from Mwaya. Thus, we contend that *T. variabilis* as it is now defined is comprised of different species.

## IMPACT, RELEVANCE, AND TECHNOLOGY TRANSFER

Jay R. Stauffer, Jr. collected fishes in Lake Malaŵi. Some of these fishes were preserved in 10% formalin, while others were placed in 95% ethanol for future genetic analyses. A series of brooding cichlids along with their young were frozen in liquid nitrogen for paternity analysis. Underwater videos of courtship behavior of many lek-breeding fishes were taken. Jeremy Likongwe, an instructor at the University of Malaŵi continued his research at Penn State. It is expected that he will complete all of the requirements for his Ph.D. during fall 1995.

Jay Stauffer continued his work to aid in the design of a study to assess the impact of over fishing on the sand-dwelling fishes in the vicinity of Lake Malaŵi National Park. Mr. O. V. Msiska's (Department of Research and Environmental Affairs, Lilongwe, Malaŵi) Ph.D. dissertation was submitted and approved by his graduate committee of which J. R. Stauffer is chair.

Collections are continuing to be deposited into the University of Malaŵi's fish museum,

which was initiated in 1991 by J. R. Stauffer. Mr. Lilongwe has been trained in the operation of the image analyses equipment and is continuing his studies at Penn State. J. R. Stauffer aided in a project undertaken by the Centers for Disease Control, Atlanta, Georgia to assess the increase incidence of bilharzia in Malaŵi.

In the summer of 1991, a laboratory was established at Chancellor College for conducting starch gel electrophoresis and three Malaŵians were trained in electrophoretic techniques. Equipment for the laboratory included a 30L cyropreservation container for freezing tissue in liquid nitrogen. The use of liquid nitrogen eliminates the need for keeping fishes alive for extended periods of time if extensive travel is required for their collection. A Beckman microfuge and a Sero-fuge centrifuge were provided for the preparation of tissue for electrophoresis. A digital pH meter, Mettler analytical balance, a top loading balance, and stir plate were provided for the preparation of reagents and buffers. Equipment for conducting the actual electrophoresis was also provided and included four power supplies, gel boxes, platinum electrodes, and staining equipment.

In addition to the above equipement, we also supplied chemicals specific for the electrophoresis buffers and staining solutions. Enough chemicals were provided for approximately 500 analyses. Most of the chemical were obtained from Sigma Chemical Company and can be reordered by technicians at the University of Malaŵi.

One month was spent training three Malaŵians in the techniques of starch gel electrophoresis, including dissection and removal of tissue from fishes, homogenization and preparation of tissue, pouring and running the gels, staining the gels, and the interpretation and analysis of results. All three of the technicians were able to use these thechniques on their own begore we left and seemed ocnfident in their ability to continue this work.

## PROJECT ACTIVITIES/OUTPUTS

A list of published and submitted papers that acknowledge this support follows.

Kellogg, K.A., J. Markert, J.R. Stauffer Jr. and T.D. Kocher 1994. Microsatellite variation demonstrates multiple paternity in lekking cichlid fishes from Lake Malaŵi. Proc. R. Soc. London. B 260:79-84.

McKaye, K. R., D. E. Mughogho, and J. R. Stauffer Jr. 1994. Sex-role differentiation in the feeding and defense of young by a biparental catfish, *Bagrus meridonalis*. Animal Behaviour 48:587-59.

Stauffer, J. R., Jr. 1994. A new species of *Iodotropheus* (Teleostei: Cichlidae) from Lake Malaŵi. Ichthyological Explorations of Freshwaters 5:331-344.

Bowers, N. J., J. R. Stauffer, Jr. and T. D. Kocher. 1994. Intra- and interspecific mitochrondrial DNA sequence variation within two species of rock-dwelling cichlids (Teleostei: Cichlidae) from Lake Malaŵi, , Africa. Molecular Phylogenetics and Evolution 3:75-82.

Stauffer, J.R., Jr. and S.S. Chiotha. 1993. Intra-lacustrine fish introductions--a threat to Lake Malaŵi Fish. Proceedings of the First Research and Development Conference. Bunda College, University of Malaŵi, Lilongwe, Malaŵi 162-164.

Stauffer, J.R. Jr., T.J. Lovullo, and K.R. McKaye. 1993. Three new sand-dwelling cichlids from Lake Malaŵi, Africa, with a discussion of the status of the genus *Copadichromis* (Teleostei: Cichlidae). Copeia 1993: 1017-1027.

McKaye, K. R., J. H. Howard, J. R. Stauffer, Jr., R. P. Morgan II, F. Shonhiwa. 1993. Sexual selection and the genetic relationships of a sibling species complex of bower building cichlids in Lake Malaŵi, Africa. Japanese Journal of Ichthyology 40:15-21.

Kocher, T.D., J.A. Conroy, K.R. McKaye, J. R. Stauffer, Jr. 1993. Extreme morphologies of

Cichlid Fish in Lakes Tanganyika and Malaŵi are due to convergence. Molecular Phyologeny and Evolution 2:158-165.

Bowers, N. J. and J. R. Stauffer, Jr. 1993. A new species of rock- dwelling cichlid (Pisces: Cichlidae) from Lake Malaŵi, Africa, with comments on *Melanochromis vermivorus* Trewavas. Copeia. 1993:715-722.

Stauffer, J. R. Jr. 1993. A new species of *Protomelas* from Lake Malaŵi, Africa. Ichthyological Explorations of Freshwaters 4:343-350.

Bowers, N.J. 1993. A revision of the genus *Melanochomis*. (Teleostei: Cichlidae) from Lake Malawi, Africa, using morphological and molecular techniques. Ph.D. thesis, Penn State University.

McKaye, K.R., D. Mughogho, T.J. LoVullo. 1992. Formation of the selfish school. Environmental Biology of Fishes. 35:213-218.

LoVullo, T.J., J.R. Stauffer, Jr., and K.R. McKaye. 1992. Diet and growth of a brood of *Bagrus meridonalis* Gunther (Siluriformes: Bagridae) in Lake Malaŵi, Africa. Copeia 1992: 1084-1088.

Stauffer, J. R., JR., Jr. and E. Hert. 1992. *Pseudotropheus callainos*, a new species of mbuna (Cichlidae) with analyses associated with two intralacustrine transplantations in Lake Malaŵi, Africa. Ichthyological Explorations of Freshwaters.

Kocher, T.D. 1992. PCR, direct sequencing, and the comparative approach. PCR Methods and Applications 1:217-221.

McKaye, K.R. 1991. Sexual selection and the evolution of the cichlid fishes of Lake Malaŵi, Africa. In: M. Keenleyside (ed.). Behavior, Ecology, and Evolution of Cichlid Fishes. Chapman, Hall, London, pg. 241-257.

Chiotha, S.S., K.R. McKaye, and J.R. Stauffer, Jr. 1991. Prey handling in *Trematocranus placodon*: a snail-eating fish from Lake Malaŵi Ichthyological Exploration of Freshwaters 2:203-208.

Chiotha, S.S., K.R. McKaye, and J.R. Stauffer, Jr. 1991. Use of indigenous fishes to control schistosome snail vectors. Biological Control 1:001-004.

Stauffer, J. R., Jr. 1991. Description of a facultative cleanerfish (Teleostei: Cichlidae) from Lake Malaŵi, Africa. Copeia. 1991(1):141-147.

McKaye, K.R., S.M. Louda and J.R. Stauffer, Jr. 1990. Bower size and male reproductive success in a cichlid fish lek. American Naturalist 35:592-613.

**Manuscripts in press and in review supported by USAID funding.**

Bowers, N., J.R. Stauffer Jr., and T.D. Kocher. in review. Inferring evolutionary relationships of closely related cichlids (Cichlidae: Teleostei) from Lake Malaŵi, Africa, using morphological and molecular techniques. Systematic Biology.

Kocher, T.D., J.A. Conroy, K.R McKaye, J.R. Stauffer, S.F. Lockwood. in review. Evolution of NADH dyhydrogenase subunit 2 in East African cichlid fish.

Stauffer Jr., J.R.. N.J. Bowers, T.D. Kocher, and K.R. McKaye. in press. Hybridization between *Cynotilapia afra* and *Pseudotropheus zebra* (Teleostei: Cichlidae) following an intra lacustrine introduction. Copeia.

Stauffer, J. R. Jr., N.J. Bowers, K.R. McKaye, and T.D. Kocher. in press. Evolutionary Significant Units among Cichlid Fishes: The Role of Behavioral Studies. American Fisheries Society Symposium 17. 54 pp. 9 figures. 1 table.

Stauffer, J. R., Jr. in press. Designation of a neotype for *Iodotropheus sprengerae* (Teleostei: Cichlidae). Bull. Zool. Nomen.

Bowers, N., J.R. Stauffer Jr., and T.D. Kocher. 1993. Inferring evolutionary relationships of closely related cichlids (Cichlidae: Teleostei) from Lake Malawi, Africa, using morphological and molecular techniques. (submitted)

In preparation:

Markert, J., N. Bowers, J.R. Stauffer Jr. and T.D. Kocher. 1994. Differentiation of microsatellite markers among populations of Melanochomis (in preparation).

Kocher, T.D., J.R. Stauffer Jr., K.J. Gilbert and K.R. McKaye. 1994. Mitochondrial DNA haplotype frequencies among populations of the lek-breeding cichlid, Copadichromis eucinostomus. (in preparation).

## FUTURE WORK

Several manuscripts have been completed and will be submitted to journals in the near future (see attached documents). J. R. Stauffer will be in Malaŵi from January - April 1995 on a Fulbright Research Fellowship.

## LITERATURE CITED

Atchley, W. R. 1978. Ratios, regression intercepts, and the scaling of data. Syst. Zool. 27:78-83.

Bookstein, F., B. Chernoff, R. Elder, J. Humphries, G. Smith, and R. Strauss. 1985. Morphometrics in evolutionary biology. The Acad. Nat. Sci. Phila. Spec. Publ. 15.

Humphries, J. M., F. L. Bookstein, B. Chernoff, G. R. Smith, R. L. Elder, and S. G. Poss. 1981. Multivariate discrimination by shape in relation to size. Syst. Zool. 30:291-308.

McKaye, K. R. 1991. Sexual selection and the evolution of the cichlid fishes of Lake Malaŵi, Africa. pp. 241-257. In M. Keenleyside (ed.). Behavior, Ecology, and Evolution of Cichlid Fishes. Chapman Hall, London.

Mosimann, J. E. and F. C. James. 1979. New statistical methods for allometry with application to Florida red-winged blackbirds. Evolution 33:444-459.

Oxnard, C. E. 1978. One biologist's view of morphometrics. Ann. Rev. Ecol. Syst. 9:219-241.

Reyment, R. A., R. E. Blackish, and N. A. Cambell. 1984. Multivariate Morphometrics, (2nd ed.) Academic Press. NY.

Stauffer, J. R., Jr. 1991. Description of a facultative cleanerfish (Teleostei: Cichlidae) from Lake Malaŵi, Africa. Copeia 1991:141-147.

Stauffer, J. R., Jr. 1993. A new species of *Protomelas* (Teleostei: Cichlidae) from Lake Malaŵi, Africa. Ichthyol. Explor. Freshw. 4: 343-350.

Stauffer, J. R., Jr., and J. M. Boltz. 1989. Description of a new species of Cichlidae from Lake Malaŵi, Africa. Proc. Biol. Soc. Wash. 102:8-13.

Stauffer, J.R. Jr., T.J. Lovullo, and K.R. McKaye. 1993. Three new sand-dwelling cichlids from Lake Malaŵi, Africa, with a discussion of the status of the genus Copadichromis (Teleostei: Cichlidae). Copeia 1993: 1017-1027.

Strauss, R. E. 1980. Genetic and morphometric variation and the systematic relationships of eastern North American sculpins (Pisces: Cottidae). Unpubl. PhD. Thesis, The Pennsylvania State University Park, PA.