PN-ABW-779
96275

11.204

FINAL REPORT

(1991-1994)

Submitted to the Office of the Science Advisor
U. S. Agency for International Development

Determination of Biodiversity in Complex Ecosystems

J. R. Stauffer, Jr.
Penn State University

T. D. Kocher
University of New Hampshire

Project Number: 11.204
Grant Number: DHR-5600-G-00-1043-00

A. I. D. Grant Project Officer: Dr. John A. Daly

REC'D IN R&D/R
AUG 17 1995

CC:DOSTID

Executive Summary

Approximately 90 species have been collected representing approximately 30 genera. Twenty-five new species have been described (see attached list of publications) and a revision of the *Tramitichromis lituris* species complex, *Pseudotropheus zebra* species complex, and the *Petrotilapia tridentiger* species complex is currently being completed. The use of behavioral characteriscs, morphological variation, and genetic analyses will be used for the species descriptions.

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 (1) to use variation in behavioral, morphological, and genetic data to delineate reproductively isolated groups of fishes and to develop low-cost techniques to identify the various stocks. We wanted to test whether population-specific variation existed in the mitochondrial DNA of Lake Malawi cichlids. Preliminary data indicated sequence differences among some species, but it was not clear that mtDNA could distinguish among very closely-related species. Second, we proposed to isolate novel hyper variable loci

from the nuclear genome of these species. Simple-sequence loci (consisting of many copies of a sequence such as CACACA...) are expected to accumulate variation more rapidly than mtDNA, and thus be useful for distinguishing closely-related species.

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*.

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* differ 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 Vua (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.

Protomelas dejunctus was described from Chinyankwazi and Chinyamwezi islands, located in the southeast arm of Lake Malaŵi. Although morphologically similar to *P. fenestratus* and *P. taeniolatus*, *P. dejunctus* can be distinguished using sheared principal component analysis of morphometric and meristic data (Stauffer 1993:348, Fig. 6), the greater number of gill rakers on the first gill, its greater head depth, the intense blue coloration of breeding males, and the presence of tricuspid teeth in its outer row of the lower jaw.

A new species of *Iodotropheus* was described from Boadzulu Island, Lake Malaŵi, Africa. *Iodotropheus sprengerae* Oliver and Loiselle, the type species of *Iodotropheus* was based solely on aquaria-raised specimens thought to have originated from brood stock collected from Boadzulu Island. Subsequently, *Iodotropheus stuartgranti* Konings was described from the eastern shore of Lake Malaŵi near the Mozambique border. A more detailed analysis of the morphometric and meristic data, however, demonstrated that the type series has closer affinities to wild-caught specimens from Chinyankwazi and Chinyamwezi islands. The new species is distinguishable from the type series and wild-caught specimens from Chinyankwazi and Chinyamwezi islands of *I. sprengerae* and the type series of *I. stuartgranti* based on shape, oral dentition, and pharyngeal-bone dentition. In order to preserve the name *I. sprengerae*, and hence the generic name *Iodotropheus*, I petitioned the International Commission of Zoological Nomenclature to use their plenary powers to disregard the holotype from aquarium-raised fish and to designate a neotype from wild-caught specimens from Chinyankwazi Island.

We have investigated mtDNA variation in the two major groups of species in Lake Malawi: the rock dwelling mbuna and the sand-dwelling utaka. Our results for each group are described below. We have also extended our analysis to include nuclear-encoded micro satellite

loci. Preliminary results for two loci demonstrate high levels of variation that will be useful for the analysis of population structure.

Mitochondrial DNA variation in Melanochromis

An extensive study of a group of rock-dwelling fish (mbuna, genus *Melanochromis*) has been completed. This genus of rock-dwelling fish includes two species complexes (*M. auratus* and *M. heterochromis*) each of which contains a number of closely-related populations. Some of these populations can be distinguished morphologically, suggesting that each population may be a unique evolutionary lineage.

Sequences of the first half of the control region were determined for 146 individuals representing 14 populations. In only one instance were multiple haplotypes observed within a single population. Interspecific divergence ranged from 0 to 2.5%. This degree of differentiation, particularly the lack of shared mtDNA types among populations, suggests that mtDNA sequences are a useful too for organizing the biodiversity of this complex ecosystem. It should also be a useful tool for assessing gene flow among populations.

Mitochondrial DNA variation in sand dwelling cichlids

Our success in identifying population-specific variants of rock-dwelling cichlids encouraged us to examine several sand-dwelling species with the same techniques. We have studied four leks of *Copadichromis* spp. from the Cape Maclear region of Lake Malawi . Three of these populations have been described as distinct species (Stauffer et al. 1993), and the fourth likely represents an undescribed taxon. The Thumbi West Island lek is small (30 bowers) and in deep water (20m). The Cape Maclear beach lek (*C. conophorus*) contains thousands of bowers, and is in relatively shallow water (6m). These two populations are separated by about 1 km of

open lake. The third population, at Kanchedza Island (*C. cyclicos*), is of moderate size, in 6m of water, about 20 km around the Cape Maclear peninsula. The final population (*C. thinos*), is found at Mazinzi Reef, about 2km from Kanchedza Island. We sequenced the control region of approximately 20 individuals from each site, and counted the frequency of different mitochondrial haplotypes at each location:

Mitochondrial Type	Locality			
	TI	CM	KI	MZ
A	16	1	0	2
B	3	11	0	3
C	0	0	14	0
D	0	4	1	2
E	0	2	1	1
F	0	1	0	0
G	0	0	2	0
H	0	0	0	1
I	0	0	0	4
J	0	0	0	1
K	0	0	0	1

These populations display significant differences in haplotype frequency, despite their geographic proximity. Unlike the *Melanochromis* species we have studied, each population does contain

several haplotypes. Also unlike *Melanochromis*, some of these haplotypes differ by multiple nucleotide substitutions. The greater heterogeneity within populations of *C. eucinostomus* could be due to maintenance of ancestral variation in these populations, which may be larger than the *Melanochromis* populations. The heterogeneity might also arise because of frequent migration and hybridization among populations. It is also possible that our collections, while tightly circumscribed in time and space, still represent a mixture of several genetically distinct populations. Additional sampling through the breeding season, accompanied by detailed behavioral observations, would help to distinguish these hypotheses. Last year, we reported the results of screening a phage library with a probe for CA-repeats. We isolated a number of positive clones, and developed primers for four loci which showed long stretches of the repetitive element. Two of these loci have amplified well, and can be scored using fluorescent detection technologies on an automated DNA fragment analyzer.

An initial survey of several mbuna and utaka populations has revealed spectacular variation for these loci. Heterozygosity for each locus is greater than 90%. In a population of *Melanochromis auratus* we identified 10 alleles in 12 individuals. Current efforts are directed at quantifying allele frequency differences among populations.

IMPACT, RELEVANCE, AND TECHNOLOGY TRANSFER

Jay R. Stauffer, Jr. collected fishes during the spring in every year, including a four month stay in 1995, which was supported by a research Fulbright fellowship. Some of these fishes were preserved in 10% formalin, while others were placed in 95% ethanol or liquid nitrogen for future genetic analyses. A series of brooding cichlids along with their young were also 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.

PROJECT ACTIVITIES/OUTPUTS

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

McKaye, K. R., J. C. Ryan, J. R. Stauffer, Jr., L. J. L. Perez, G. I. Vega, E. P. van den Berghe. 1995. African tilapia in Lake Nicaragua: Ecosystem in Transition. BioScience 45:406-411.

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. and J. R. Stauffer, Jr. in press. Nine new species of rock-dwelling cichlids (Teleostei: Cichlidae) from Lake Malaŵi. Ichthyological Explorations of Freshwaters.

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).

LITERTATURE 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.