

Genetic Diversity of a Lake Victoria Cichlid: *Haplochromis Piceatus*

Katelyn M. Bonko and Erin McMullin*

Biological Sciences - Bowling Green State University, Bowling Green, Ohio 43403



INTRODUCTION

Lake Victoria, bordering Uganda, Tanzania, and Kenya, is Africa's largest freshwater body. The lake has cycled through three drying periods, filling for the last time approximately 14,700 years ago (Verheyen et al, 2003). The cichlid population since that time has diversified into 500 species, making Lake Victoria a biological hotspot for freshwater organisms. In the 1950s the Nile Perch was introduced to boost fisheries with an explosion of the species in the 1980s. Of the 123 cichlid species originally caught at sampling stations in the Mwanza Gulf, Tanzania, 80 had disappeared from the catches in 1986 due to domination of the Nile Perch (Witte et al, 1992). Furthermore, extrapolating the data from the Gulf across Lake Victoria implies that "approximately 200 of the 300+ endemic haplochromine species have already disappeared" (Witte et al, 1992).

Microsatellites are able to incorporate large ecological issues into small scale, genetic approaches by focusing on nucleotide repeats in a genome. Through polymerase chain reaction (PCR), specific primers can identify flanking regions and amplify microsatellite loci of interest (Selkoe & Toonen, 2006). Through the use of fluorescently labeled primers, we were able to go beyond differentiating the crude estimate of allelic length and determine the length of each allele. The overall goal of the project is to 1) Determine the overall genetic diversity of the *Haplochromis-Piceatus* population in the Toledo zoo and, 2) determine if (and how much) genetic diversity is decreasing within the population.

Figure 1: *Haplochromis Piceatus*. Image courtesy of: http://aquarium-webzine.com/images/victoria/piceatus/hp_sylvain-dubreuil-00001.



MATERIALS/METHODS

The Toledo Zoo has four generations (F7-F9) of *H. Piceatus*; fin clips were taken from the dorsal fins of twenty individuals from each generation, for 60 samples total. The samples were obtained by Jay Hemdal and preserved in 0.5 mL of RNAlater solution before DNA extraction. DNA extractions were performed with the standard phenol-chloroform technique. Samples were suspended in 100 μ L water, diluted 1:20 and stored in 4°C. Polymerase chain reaction (PCR) was performed by preparing a cocktail: 2 μ L 10x buffer, 2 μ L dNTPs mM, 1 μ L forward primer (labeled), 1 μ L reverse primer, 0.25 μ L Taq, 13 μ L water, 1 μ L of DNA, and 1 drop mineral oil. PCR products were sent to the Iowa State DNA facility (www.dna.iastate.edu) for fragment analysis on an Applied Biosystems 3100 Genetic Analyzer. Data were viewed using *Peak Scanner v. 1* (Applied Biosystems), and each PCR product was individually assessed for the tallest peak coordinating to the allele of interest.

Primer	Anneal Temp.	Dye
PMV13	52°C	HEX
PMV4	52°C	FAM
HST1	56°C	FAM
HST68	56°C	FAM
HST53	56°C	HEX
HST44	55°C	HEX
PPUN17	57°C	FAM
HST6	56°C	FAM
PPUN7	57°C	FAM
PPUN20	57°C	HEX
PPUN9	57°C	FAM
PPUN21	57°C	HEX

Figure 2: Chart of Primers, Annealing temperatures, and fluorescent dye.

Applied Biosystems Software: Peak Scanner v. 1

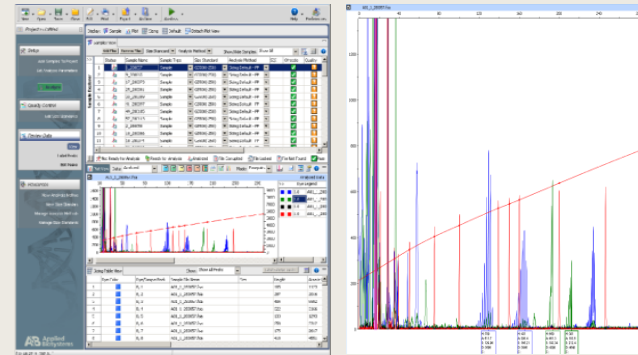
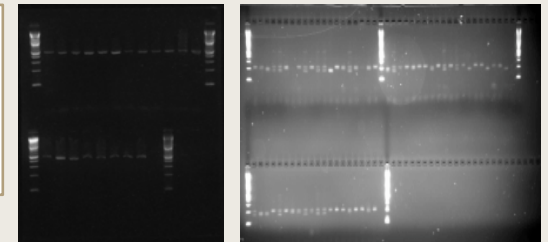


Figure 3 (Far left): Main screen after analyzing samples with software. The tallest peaks are highlighted to determine which alleles the PCR product amplified.

Figure 4 (Right): Graph from main screen enlarged to show red ladder and alleles of interest. Data from this screen is exported into Microsoft Excel for comparative analysis.

Figure 5 (left): 1% agarose gel of DNA extracted from F7 generation, Toledo Zoo. Bands of ladder on edges are, from bottom, 100bp, 200bp, 300bp, 400bp, 500bp.

Figure 6 (right): 2% agarose gel PCR product from primer set ppun9. PCR products are flanked by 100bp ladder. The bands on the gel signify the variability among the different fish. The different bands in different animals visually demonstrate genetic diversity.



Conclusions/Future Plans

Sampling kits have been sent to three other aquarium facilities with *H. Piceatus*: Paula Kolvig (Moody Gardens), Eddie Sunila (San Antonio Zoo), and Paul Loiselle (New York Aquarium). The same experimental design will be applied to these samples, in the hopes of maintaining healthy levels of genetic diversity within the surviving populations of *Haplochromis Piceatus*.

Data will be analyzed to look for genetic diversity in the Toledo Zoo samples. The data will also be used to estimate effective population size (N_e) of this population. N_e estimates the number of individuals in a given set that would "show the same amount of dispersion of allele frequencies under random genetic drift or the same amount of inbreeding" as the population set (Wiki). In addition, the Toledo Zoo samples will be compared to the *H. Piceatus* samples maintained at other institutions across the United States; this will further the objective to compare genetic diversity in the separate populations and to look for alleles that are unique to each population. The unique alleles are called "private alleles," which are good on an ecological scale because if the species is reintroduced into the wild, the population will be more diverse.

Acknowledgements

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