

DNA Microsatellites to Identify White Bass Stocks in Lake Erie

Jeffrey G. Miner
Ronny C. Woodruff

Department of Biological Sciences
Bowling Green State University

LEPF SG 84-98

Summary

The population of white bass *Morone chrysops* in Lake Erie experienced substantial reduction in the 1980s concomitant with the introduction of the congener white perch *M. americana* (OMNR 1995). Although white perch abundance has declined and water quality has improved in Lake Erie, white bass have not significantly increased and this is attributed to poor recruitment (ODNR 1996). To enhance management practices for restoring the white bass population it is important to understand the critical bottlenecks limiting the population. Given that white bass appear philopatric (return to their natal spawning grounds) to spawning habitats in major Lake Erie tributaries and western basin reefs, it is possible that genetic isolation among subpopulations has been sufficient to uniquely identify these subpopulations. This would be valuable to fishery biologists who could decide how to allocate efforts among subpopulations in order to optimally manage the entire Lake Erie population. To obtain initial insight into the genetic heterogeneity and uniqueness of genetic markers among western basin subpopulations of white bass, we sampled spawning fish from the Maumee and Sandusky rivers and conducted microsatellite analyses. Only one of three primer sets revealed any polymorphism. Additional surveying of primers will be necessary to identify a larger suite of microsatellites with allelic polymorphism before statistical analyses can be conducted to determine if there are unique haplotypes among subpopulations.

Background

White bass is an important sportfish in Lake Erie that uses tributaries (and reefs) in which to spawn, but the population size has been reduced substantially from unidentified mechanisms including fishing pressure, introduced species (white perch), and changes in water quality. Because this fish spawns in tributaries, reproductive isolation among spawning subpopulations may have led to genetic differences that can be detected (Avisé 1994; Leclerc et al. 1996; Diaz et al. 1998). Given the limited funds necessary to manage Lake Erie sport fish populations, knowing the proportion of the Lake population that comes from each tributary would be a valuable asset. If subpopulations can be clearly identified, this technique could help fishery biologists begin to pinpoint strategies for either preserving the most viable spawning locations or restoring degraded systems.

Procedures used to detect isolated subpopulations have developed rapidly in the 1980s and 1990s, from the use of allozymes to suites of DNA analyses. The use of restriction fragment-length polymorphism (RFLP, DNA fingerprinting) is a standard approach to determine both kinship (e.g., paternity) and intraspecific phylogeny (Avice 1994). In general terms what is done is that an enzyme (i.e., primer or restriction endonuclease) is used to cleave DNA at a particular nucleotide sequence, usually four to six base pairs in length. Once these primers have been obtained (from bacteria) they are used to cleave (restrict) DNA. The resulting DNA fragments are separated according to their molecular weight by gel electrophoresis and then visualized by varying techniques. Specifically, we conducted a preliminary study using DNA microsatellites to identify polymorphic regions that could be used to discriminate among subpopulations. Microsatellites are regions of DNA consisting of reiterated short sequences (2-4 nucleotides) tandemly arrayed. These regions are assumed to be neutrally selected and thus mutations occurring in isolated subpopulations will persist. For example, one subpopulation may have many individuals with a microsatellite region with six repeats of a particular nucleotide pattern (e.g., GTAA), while another population is dominated by individuals with eight repeats. Polymerase Chain Reaction with RFLP using microsatellites would enable the detection of these differences because of the different molecular weights of the DNA stands with either six or eight repeats. If all individuals from one subpopulation had only six repeats at a locus while all individuals from another subpopulation had only eight repeats, then primers for this region of DNA alone would be sufficient to discriminate individuals from the two subpopulations. However, it is much more common for subpopulations to share alleles (numbers of repeats), but for the frequency of each allele to vary among subpopulations (if there is any frequency difference at all). The presence of unique alleles and frequency differences can be used to infer the presence of distinct subpopulations. It is typical that between three and 10 loci (usually with unique primers) with varying degrees of polymorphism are needed to determine whether or not subpopulations are significantly different.

Objectives and Approaches

Developing primers for analysis of microsatellites is laborious and expensive. However, it is not uncommon for primers that work successfully to identify polymorphic regions of DNA on one species to also work with similar taxa. In this small grants exploratory project, we set as our goal to determine if primers that have been used with striped bass (*M. saxatilis*), a congener of white bass, could be used to identify polymorphic microsatellites in white bass. In addition, we wanted to determine if we could identify any potential differences in the allelic frequencies between several populations of white bass in Lake Erie. Although it can take a hundred or more fish from each subpopulation to determine allelic frequencies, we chose in this exploratory study to obtain 20 white bass from each of three potential subpopulations: the Maumee River, the Sandusky River, and Lake Erie spawning white bass from the reefs in the western basin. Primers for three striped bass microsatellite loci were obtained from Dr. Bert Ely (University of South Carolina).

Locus	Primers	Size (bp)	PCR Annealing Temp (C)
SB83	FT38, FT36	~160	50
SB6	FT87, FT88	~ 200	46
SB8	BE341, BE342	~ 210	46

Results and Conclusions

We were able to use striped bass primers to obtain microsatellite information on the congeneric white bass from Lake Erie, although most of the project period was spent determining the exact conditions needed to get amplification of the microsatellites. Allelic polymorphism within populations was detected for only locus SB6, while SB83 was monomorphic. For locus SB8, we had to get positive controls (striped bass samples) to try to get the microsatellite to amplify and even for these controls, amplification could not be obtained. It appeared that the alleles for SB8 were different between Sandusky and Maumee subpopulations (fish from the reef subpopulation could not be obtained), but further work is necessary to state this with certainty.

Other primers are available from striped bass. Near the conclusion of this preliminary study, we received primers for eight additional loci, but did not detect loci with polymorphism. Further work with these primers and others may provide sufficient polymorphism to assess the extent of population subdivision for white bass in Lake Erie. Recent work by White (2000) using protein electrophoresis on white bass suggest that there is quite low genetic variation among white bass populations, but protein electrophoresis has much lower resolution for detecting genetic differentiation among populations than microsatellite analysis. We are confident that the approach taken here can detect subpopulation genetic variation in white bass, if in fact unique subpopulations with little gene flow exist in Lake Erie.

Student Training

One undergraduate student was trained in the procedures for DNA extraction, PCR, and electrophoresis of microsatellites. She conducted all the laboratory analyses including the running of agarose gel electrophoresis to initially screen for polymorphism. A senior graduate student was hired to supervise the undergraduate's work and run the polyacrylamide electrophoresis to obtain the final set of results. The undergraduate student is now a graduate student working on conservation biology.

References

- Avise, J.C. 1994. Molecular markers, natural history and evolution. Chapman & Hall, Inc., New York.
- Leclerc, G.M., M. Diaz, B Ely. 1996. Use of PCR-RFLP assays to detect genetic variation at single copy nuclear loci in striped bass (*Morone saxatilis*). *Molecular Marine Biology and Biotechnology* 5:138-144.
- Diaz, M., J. McPherson, B. Ely. 1998. Striped bass population subdivision within the Santee-Cooper system, South Carolina. *Molecular Marine Biology and Biotechnology* 7:191-196.

- ODNR (Ohio Department of Natural Resources). 1996. Ohio's Lake Erie Fisheries 1995. Lake Erie Fisheries Units, Sandusky, OH.
- OMNR (Ontario Ministry of Natural Resources, Lake Erie Management Unit). 1995. Lake Erie fisheries report 1993, OMNR, Wheatley.
- White, M.M. 2000. Genetic variation in white bass. Transactions of the American Fisheries Society 129:879-885.