USE OF GENETIC MARKERS TO EVALUATE PATTERNS OF DISPERSAL OF AN EXOTIC SPECIES IN ILLINOIS RESERVOIRS

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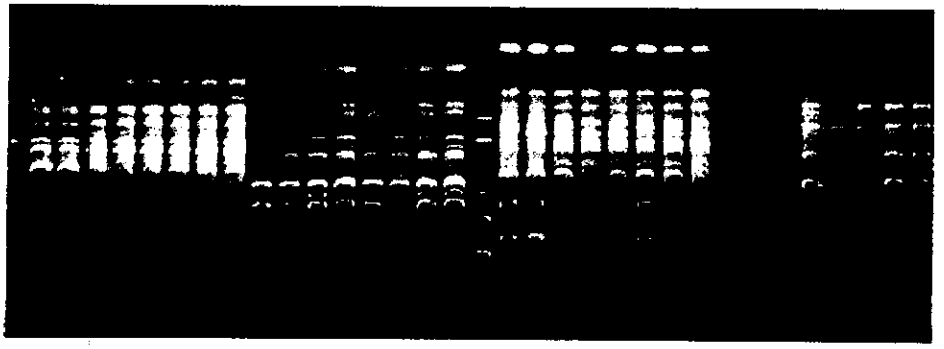
Daphnia lumholtzi (Crustacea, Cladocera), a species native to Africa, India, and Austrailia, is believed to have been introduced along with stocking of Nile perch into Fairfeild Reservoir, Texas in the early 1980's. Within two decades it has spread throughout most of the central southeastern United States. We are working to compare several populations of *D. lumholtzi* using genetic markers (ISSRs) in effort to explain the pattern of dispersal throughout their introduced range. Our work ultimately will increase knowledge about the introduction and dispersal pattern of an exotic species throughout waterways in Illinois. Enhanced understanding of mechanisms of dispersal will improve our ability to manage natural resources, to control exotic species, and protect native fish populations.

Daphnia spp. reproduce by ameiotic parthenogenesis. Natural populations consist of over 95% female, with males produced only under environmental stress. Typically, diploid eggs develop directly without fertilization. In *D. lumholtzi*, a single female may produce as many as 12 neonates in each of 12-15 broods. As a result, mass cultures of genetically identical individuals can be produced for DNA extraction.

Intersimple Sequence Repeats (ISSRs) are non-coding sequences in the nuclear genome. These hypervariable markers allow identification of distinct populations of *D. lumholtzi* because they produce a relatively high number of polymorphic loci. Once sufficient numbers were obtained in individual mass cultures, DNA was extracted. Thirteen primers - 843, 844, 17898, 17901, AW2, AW3, AW4, BECKY, DAT, GOOFY, HANS, MAO, and OMAR - were screened on DNA extracted from clonal cultures of *D. lumholtzi* isolated from Lake Taylorville, Christian County, Illinois. Gel electrophoresis revealed distinctive ISSR banding patterns in PCR (polymerase chain reaction) product generated by six of these primers (as illustrated for AW3, 844, OMAR and HANS (l.-r.) in Figure below). Similar distinct banding patterns were observed for 843 and BECKY. During summer of 2001, we are working to establish clonal cultures of *D. lumholtzi* collected from additional sites within Illinois. In addition, we intend to analyze replicate cultures from each lake to evaluate variability within a single population.



Proceeds from the Wildlife Preservation Fund were utilized to purchase various reagents necessary to conduct genetic analyses. These included nuclei lysis and protein precipitation solutions (necessary for DNA extraction) along with PCR nucleotides (for DNA amplification) and DNA standards (for gel electrophoresis). In addition, starter cultures of *Daphnia magna* were purchased for use as a surrogate species during initial of primers suitable for work on *D. lumholtzi*. Matching funds were provided by the Department of Biological Sciences for purchase of additional reagents along with requisite laboratory supplies (including agarose, *Taq*-polymerase, ethidium bromide, TBE buffer, disposable gloves, pipet tips, etc.) and minor equipment (e.g., additional "rigs" for performing agarose gel electrophoresis).

Administrative timing placed constraints on completion of this project by 30 June 2001. Our initial time line (proposed in April, 2000) specified collection of *D. lumholtzi* during May and June with production of clonal cultures during July followed by genetic analyses. Note that approval to expend funds was not received until 5 September 2000 and the agreement was not finalized until 30 October 2000. Therefore, initial efforts necessarily were limited to collections made from Lake Taylorville. However, we likely were able to screen more primers (maximizing the likelihood of ultimate success) because we focused on a single collection site. Work is ongoing and cultures have been established this season (May-June, 2001) using individuals obtained from other sites within Illinois. Genetic analyses on *D. lumholtzi* from these and other locations will proceed throughout the summer and into fall.

Nonetheless, support from the IDNR has served as a catalyst for this research initiative. Results to date were presented at the annual meeting of the Illinois State Academy of Science in April, 2001. Perhaps most importantly, the Wildlife Preservation Fund grant has served as an opportunity to mentor one undergraduate and two graduate students. One of these students has received a research scholarship from the Illinois Lake Management Association to continue work on *D. lumholtzi*. Additional funding in support of this project has been obtained in the form of a grant in aid of research from Sigma Xi, the Scientific Research Society. Finally, intramural funding has been provided as a 2001 Summer Faculty Research grant by Eastern Illinois University.