Relationship between Habitat Characteristics and the Extinction of Lake Mussels in Minnesota

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A Final Report to the Minnesota Department of Natural Resources

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Abstract- The objective of this study was to determine factors driving lacustrine mussels to local extinction by seeking patterns in the physical, chemical, biological and geographical characteristics of lakes from which they have recently disappeared or in which they are experiencing population decline. Although not foreseen in the proposal, molecular genetic methods were also used to assess genetic diversity of some populations. We studied patterns of recruitment, density, and mortality of freshwater mussels in 25 lakes, in a region of north central Minnesota where mass die-offs have been observed. Although 88 % of the lakes contained extant mussel populations, more than 40 % of them showed signs of serious decline. Recruitment rates in *Pyganodon grandis grandis* appeared lowest in small lakes with low fetch that were closest to the main river course. Recruitment in *Lampsilis radiata siliquoidea* was also low in small lakes and lakes containing populations with skewed sex ratios, but paradoxically increased with decreased abundance of potential host fish. Sex ratio (males to females) ranged from 1:6 to 4:1 in *Lampsilis* populations, and appeared to be under environmental control. The sex ratio was strongly negatively correlated with the average pH. Lake pH reduced to around 7.0 apparently led to male-biased sex ratios and recruitment failure. We also developed methods employing random amplified polymorphic DNA (RAPD), heteroduplex, and single-stranded conformational polymorphism (SSCP) analyses using nuclear DNA markers to investigate the influence of population size on heterozygosity in seven different sized populations of the freshwater mussel *Lampsilis radiata siliquoidea*. Within populations, heterozygosity values ranged from 0.161-0.356 for the RAPD loci and from 0.000-0.300 for the repeat flanking locus that formed heteroduplex molecules. There was only a weak correlation between population size and heterozygosity for the RAPD or repeat flanking locus, but the power of analyses was low due to restricted sample sizes. This result is consistent with the hypothesis that small, isolated populations may experience inbreeding. A combination of subtle environmental changes and genetic degradation may be endangering a large proportion of lake-dwelling mussel populations.

General Introduction

James Williams of the US Fish and Wildlife Service, a member of the *American Fisheries Society*'s Endangered Species Committee, has written recently: *"The high number of imperiled freshwater mussels in the United States and Canada ... portend a trajectory toward an extinction crisis that, if unchecked, will severely impoverish one of our richest components of aquatic biodiversity"* (Williams et al. 1993). Freshwater mussels are keystone organisms that are among the most endangered animal groups in the world. In spite of this, little research is performed on the threatened biodiversity of aquatic systems (Moyle and Williams 1990) or that of invertebrates (Wilson 1987), even though threats to these organisms may be proportionately greater than threats to terrestrial vertebrates (IUCN 1990; Williams et al. 1993). Freshwater mussels are among the most endangered animal groups in the world (Fig. 1): 72% of all North American mussel species are rare or endangered (Williams et al. 1993; *cf.* 17% of mammals, 12% of birds, 4% of reptiles and amphibians; IUCN 1990). More than 7% of mussel species may already be extinct.

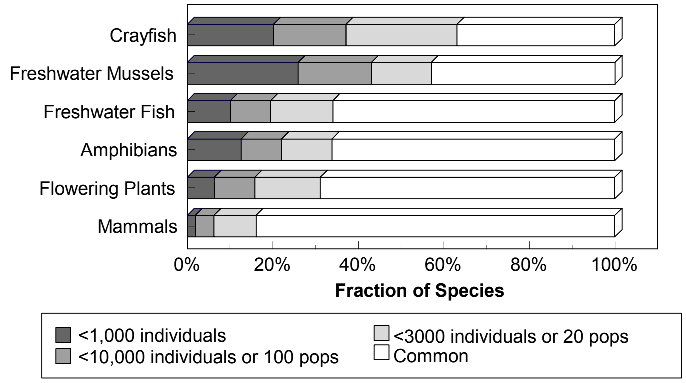

Fig. 1. Scorecard of scarcity according to the *Nature Conservancy* and the *Natural Heritage Network* (1994)

The extinction process can be viewed as the successive extinction of local populations, until the extinction of the last of them renders the species, as a whole, extinct. There is therefore no fundamental difference between the process giving rise to extinction of local populations and that extinguishing a species. There is growing evidence of mass die-offs of mussels in lakes which, if unchecked, must eventually lead to the elimination of mussels from the lacustrine biota.

A number of lines of evidence suggest that the local extinction of lake mussel populations is an increasingly common phenomenon in Minnesota and elsewhere. Neves (1987) reports mass die-offs of mussels in lakes in Michigan and New York that may have been associated with stress (pesticides, weather, etc.). Fleming et al. (1995) report that the frequency of mass die-offs in lakes and streams is increasing rapidly. A study of 70% of Iowa's natural lakes shows that mussels are now extinct in all lakes surveyed (Jeff Straka, Iowa State University, unpublished). Surveys of lakes in Quebec and northern Minnesota during 1990-1995 revealed that mussels have fallen to local extinction in many of them (J.A. Downing, ISU and W.L. Downing, Hamline University, unpublished). In the 1930s, numerous freshwater mussels were found in Wayzata Bay of Lake Minnetonka (Wood 1938) but in 1993 dead shells, but no living organisms, could be found (Downing, unpublished). Finally, the entire population of Wabana Lake, a pristine lake in Itasca County (Downing et al. 1992; Downing and Downing 1993) was extirpated by unknown causes during the summer of 1995 (J.A. Downing, unpublished). It is therefore likely that Minnesota's lake mussels are proceeding toward extinction by the accelerating disappearance of entire populations.

Mussels are an important part of the lake biota. Freshwater mussels often dominate the benthos in lakes (Golightly and Kosinsky 1981). They are large, selective suspension feeders and impact phytoplankton (Price and Schiebe 1978, Libois 1988). Their pseudo-feces enrich sediments (Downing 1991) stimulating the production of other invertebrates (Sephton et al. 1980). They dominate alkalinity budgets of lakes (Green 1980). They are food for fish and mammals (Cvancara 1970), and their larvae are fish food and sometimes fatal parasites (Matteson 1948). They are also of paleontological and archaeological interest (Green 1972). There is an urgent need to seek factors leading to their extinction.

Background and Literature Review

Mussels' biology may contribute to their susceptibility to extinction. Reproduction is a very critical stage in freshwater mussels. Fertilization success is rarely complete (Lefevre and Curtis 1910; Downing et al. 1993). Fertilized eggs develop into larval "glochidia" that are released to parasitize specific host fish especially yellow perch (*Perca flavescens*), but also *Lepomis macrochirus*, *Micropterus* spp., *Pomoxis annularis*, *P. nigromaculatus*, *Roccus chrysops*, *Stizostedion* spp. and others. Glochidia parasitize fish for a month or so before falling from the fish as juveniles. Juveniles must settle immediately in suitable substrate. Any process interfering with adult mussels, the production of viable larvae or the ecology and behavior of parasitized fish will therefore impact mussel populations.

Several explanations for the rapid decline in mussel populations and species have been postulated. Mussels are very long-lived (Bauer 1992), mature slowly and become reproductively senescent at about 60% of their maximum body size (Downing et al. 1993). Therefore, mussels may become functionally extinct long before the death of the last individual (Bogan 1993), since reproduction depends upon gamete formation and fish host availability. Because of mussels' long lives, minor impacts may become cumulative and lethal with the passage of time (Nalepa et al. 1991).

Four important causes for the widespread extinction of mussels have been proposed (Bogan 1993): habitat destruction, lack of host fish, commercial over-exploitation, and competition from introduced species. These include all of Diamond's "evil quartet" (Diamond 1984, 1989). Principal sources of habitat destruction are siltation from channel modification, poor agricultural practices, clear-cutting, pollution from municipal and industrial sources, acidification, sediment modification, and pesticide and heavy metal pollutants (Bogan 1993). Any perturbation of the host fish must also have serious implications for mussel reproduction (Fuller 1974). Mussels have been and are still exploited actively and exploitation has been associated with population extirpation in Europe (Young and Williams 1983). Exotic bivalve species such as the zebra mussel (*Dreissena polymorpha)* and the Asian clam (*Corbicula fluminea*) compete with native mussels, and are associated with their disappearance in most invaded sites (Clarke 1988; Hebert et al. 1991; Mackie 1991). Mussels are sensitive to concentrations of suspended particulate matter, and only rarely live in lakes with high silt content (Bogan 1993). They also have several important parasites, and at least one mass die-off in a Minnesota lake has been accompanied by heavy infestations of parasitic mites (*Unionicola* sp.) and necrophagous protozoa (Downing in prep.). Changes in the physical/chemical environment, as well as changes in aquatic community composition, may cause the decline of freshwater mussel populations.

Fragmentation of populations may also be a factor. Lake-dwelling mussel populations have been broken into small fragments by dam construction over the last 50-100 years (Watters 1996). Population viability theory suggests that small population fragments can be endangered by their smallness *per se*. The small population fragments arising due to decreased movement of glochidia-bearing fish among lakes may have increased inbreeding leading to lowered fitness and survival (Soulé 1987). Almost no attention has been given to this *small-population paradigm* (*sensu* Caughley 1994) as an explanation for their disappearance (Bogan 1993, Williams et al. 1993). In other bivalves, however, there is a long-standing literature on the relationship between genetic variation and fitness parameters (Allendorf and Leary 1986, Zouros et al. 1980, David et al. 1995). Existing work on the population genetics of freshwater mussels suggests that isolated populations have lowered heterozygosity (Kat 1982).

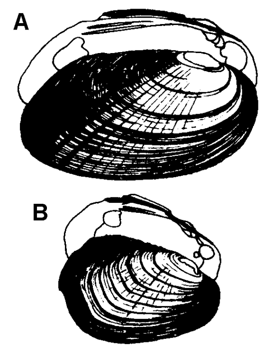

Fig. 2. *Lampsilis radiata siliquoidea* (A is male, B is female) from Harman (1970). Shells can be as much as 12 cm in length (Clarke 1973).

Location of Study

The study was carried out in Itasca and Cass Counties. Our original proposal sought to contrast urban and more pristine settings, but this was abandoned due to funding constraints. Instead, we concentrated on an area of Minnesota experiencing mass die-offs and sampled 40% more sites than originally proposed. We also added an exploratory molecular genetic component to the study.

Methods and Materials

Although specific methods are presented in the theses and submitted manuscripts that are attached to this report, we tested the general hypothesis that extinction of mussel populations has been a random process, against the alternate hypothesis that extinction has been most frequent in impacted ecosystems or those that presented small population sizes.

We establish a matrix of lakes that represented patches of different sizes. We used existing chemical and physical data to restrict our study to only those lakes presenting nominally suitable habitat for freshwater mussels (e.g. no "acid" lakes, no lakes without suitable sedimentary substrate). Lake size categories were initially 10-50 ha, 50-500 ha, 500+ ha, but some adjustments were necessary to conform to available lake combinations in the region. In the end, we sample 25 individual target lakes (only 18 were proposed in our original study that was only 50% funded) representing different sizes of habitat patches.

At each of the study lakes, we searched for evidence of shells on land around lakes, as well as empty shells and living organisms in shallow water. Lakes were assigned to three categories: (A) lakes where mussels have not lived over the last ca. 50 years, (B) lakes where mussels have lived but are now extinct, and (C) lakes where mussels have lived and are currently extant. Initially we had planned to combine these observations with a suite of habitat descriptors to determine which of the lake characteristics are best associated with recent extinction. In the end, 88% of our study lakes contained extant mussel populations, so measures of density, recruitment, mortality and sex ratio were used to gauge the relationship between population health and local habitat characteristics.

Mussel population status was determined by searching shore and shallow water for empty mussel shells and living animals (*see* attached manuscripts for a more complete description of methods). Searching was concentrated around sites in the lake receiving sufficient wave energy to produce sandy sediments. Lakes were thus assigned to one of three categories: (A) mussel-free, (B) recently extinct, or (C) extant. Although preliminary surveys suggested that about 20% of the lakes visited would fall into category A, in reality only about 12% of lakes containing suitable substrate lacked extant mussel populations.

The condition of extant populations was determined through analysis of life and death assemblages, size distributions as surrogates of age distributions, and by analysis of sex ratios of living populations (*see* sexual dimorphism of *Lampsilis* in Fig. 2). Indices of recruitment and mortality were estimated from shell length distributions and comparison of life and death assemblages, determined by collecting a minimum of 50 live and 50 dead shells, at random, at each site. Living mussels were held immersed in the lake water. The longest axis of each shell was measured with a digital caliper (± 0.1 mm). Living mussels were then returned to the substrate and empty shells were retained for further lab analyses and voucher collections. The recruitment index was calculated as the proportion of mussels < 50 mm in length in *Lampsilis radiata siliquoidea* and < 60 mm in *Pyganodon grandis grandis*. These sizes were chosen to represent comparable estimates of recent recruitment because mussels rarely become sexually mature at a length less than 50 mm, while *Pyganodon* generally grows faster than *Lampsilis*. In Prairie Lake, the recruitment index was based only on the death assemblage since it had experienced a recent mass mortality event. A mortality index was calculated as the proportion of mussels that were found dead in each lake. Life assemblage sex ratios of *Lampsilis radiata siliquoidea* were calculated as the proportion of males at the study site, ignoring individuals that could not be sexed on the basis of shell morphology. The death assemblage sex ratio was calculated as the fraction of male shells collected at the study site, also ignoring shells for which sex could not be determined.

Characteristics of mussel populations and lakes were entered into a multiple regression analysis and the factors best mussel population condition were determined by multiple correlation analysis. Factors most likely to increase extinction probability were determined as those with highest negative correlations with reproduction, mortality and density.

Because of the extreme endangerment of freshwater mussel populations and the fact that isolation of populations can be genetically disastrous, we also wished to evaluate the impact of population size on genetic variation in Minnesota lake mussel populations. We therefore performed the first such analyses of lake mussel populations using state-of-the-art molecular genetic methods.

Results and Discussion (*see also* Manuscripts Attached)

This project yielded the first detailed analyses of the relationship between mussel population characteristics and the ecological and biological condition of lakes. It also yielded the first analyses of the relationship between lake mussel population size and genetic variation in these highly isolated and endangered populations.

Although 88 % of the lakes contained extant mussel populations, more than 40 % of them showed signs of serious decline. Recruitment rates in *Pyganodon grandis grandis* appeared lowest in small lakes with low fetch that were closest to the main river course. Recruitment in *Lampsilis radiata siliquoidea* was also low in small lakes and lakes containing populations with skewed sex ratios, and increased with decreased abundance of potential host fish. A very surprising result is that the sex ratio (males to females) seemed to be under the adverse control of lake pH, ranging from 1:6 to 4:1 in *Lampsilis* populations. Lake pH reduced to around 7.0 apparently led to male-biased sex ratios and absolute recruitment failure. Although much greater sample sizes are needed to uphold this conclusion, it appears that very mild acidification of Minnesota lakes is sufficient to impact mussel populations.

In order to estimate the impact of isolation and population size on mussel populations, we also developed methods employing random amplified polymorphic DNA (RAPD), heteroduplex, and single-stranded conformational polymorphism (SSCP) analyses using nuclear DNA markers. We investigated the influence of population size on heterozygosity in seven different sized populations of the freshwater mussel *Lampsilis radiata siliquoidea*. Within populations, heterozygosity values ranged from 0.161-0.356 for the RAPD loci and from 0.000-0.300 for the repeat flanking locus that formed heteroduplex molecules. These data were similar to the ranges suggested by electrophoretic analyses. There was only a weak correlation between population size and heterozygosity for the RAPD or repeat flanking locus, but the power of analyses was low due to restricted sample sizes (we had no funding to do this). This result is consistent with the hypothesis that small, isolated populations may experience inbreeding. A combination of subtle environmental changes and genetic degradation may therefore be endangering a large proportion of lake-dwelling mussel populations in north central Minnesota.

Manuscripts Resulting from this Study

Several manuscripts resulting from this work are published as theses or are being reviewed by major journals. They are:

***Di Paolo, L.A.** 1997. Factors contributing to the local extinction of mussels: a comparative analysis of population density, recruitment and mortality. M.Sc. Thesis, Iowa State University, Ames, IA. 103 p.

***Tuxbury, K.A.** 1997. Development of DNA markers and the genetic analysis of seven populations of the freshwater mussel *Lampsilis radiata siliquoidea* (Bivalvia: Unionidae) in north central Minnesota. M.Sc. Thesis, Iowa State University, Ames, IA. 76 p.

***DiPaolo, L.A. and J.A. Downing**. A comparative analysis of mussel (Bivalvia: Unionidae) recruitment, density and mortality in a lake region experiencing mass mortality. submitted to *Canadian Journal of Fisheries and Aquatic Sciences.*

Tuxbury, K.A., B.S. Bowen, C.L. Brockhouse and J.A. Downing. Heteroduplex DNA analysis of freshwater mussels (Family Unionidae). submitted to *Molecular Ecology*

Tuxbury, K.A., B.S. Bowen, and J.A. Downing. A genetic study of seven populations of the freshwater mussel Lampsilis radiata siliquoidea in north central Minnesota. submitted to *Malacologia*.

*Articles marked with an asterisk are attached to this report and contain all the detailed methods and data resulting from this study.