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Effect of sampling effort on the efficiency of the timed search method for sampling freshwater mussel communities

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Abstract. Qualitative sampling is recommended over quantitative sampling when the objective of a mussel survey is to find rare or endangered species. Although advice on how to conduct quadrat surveys is available, there is little information on the influence of sampling effort on the effectiveness of timed searches. We conducted timed searches for mussels at 28 sites on 5 rivers in southwestern Ontario using 4.5 person-hours (p-h) of sampling effort/site. The survey period was divided into 3 equal time intervals, and the number of species and individual mussels collected within each interval were recorded. Increasing the sampling effort dramatically improved the detection of rare species. Sampling for 1.5 p-h, which is the level of effort commonly used in timed search surveys, would have resulted in <1/2 of the rare species being found and would have underestimated species richness by an average of 37%. Seventy percent of encounters with 3 federally endangered species took place during the 2nd and 3rd time intervals. Increasing the sampling effort from 1.5 to 4.5 p-h/site resulted in all but 2 of the 28 species being found at more sites. Even 4.5 p-h of effort may be insufficient for detecting all species at all sites or estimating species richness for a river. Researchers who rely on qualitative surveys to determine the presence of rare and endangered mussel species should consider the amount of time they spend at their sites because sampling effort can significantly affect their ability to detect these species.

Key words: freshwater mussels, sampling methods, endangered species, rare species.

The timed search method is generally considered to be more effective than the quadrat method for detecting rare species of freshwater mussels (e.g., Strayer et al. 1997, Vaughn et al. 1997). Quadrat sampling is preferred, however, if density or demographic information is needed. Many studies offer advice on the best sampling design to use and the number of replicates to collect when conducting quadrat surveys (e.g., Downing and Downing 1992, Green and Young 1993), but there is less guidance available on the effort needed to properly assess mussel communities using the timed search method. Strayer et al. (1997) found that 1-h timed searches were capable of detecting even sparse populations of mussels (<0.01/m²) in small streams. Hornbach and Deneka (1996), sampling a large river, found that they needed to collect between 100 and 700 mussels/site to identify most of the species present. Recent timed searches generally used a fixed sampling effort of 1 to 2.5 personhours (p-h)/site (Mackie 1996, Strayer et al. 1997, Vaughn et al. 1997, Morris and Di Maio 1998), or a variable sampling effort that was site-dependent (e.g., Clarke 1992 averaged 2.3 p-h/site, with a range of 0.4–8.0 p-h; Obermeyer 1998 averaged 1.4 ± 0.3 SD p-h/site).

Our research in recent years has focused on the assessment of rare species of mussels in southwestern Ontario rivers (Metcalfe-Smith et al. 1998, 1999). Our specific goals were to determine if species known only from historical records were now extirpated, if other rare species were declining in abundance or experiencing range reductions, and if species richness had changed over time on a site-to-site or river-wide basis. We therefore chose a relatively intensive sampling effort of 4.5 p-h for our timed search

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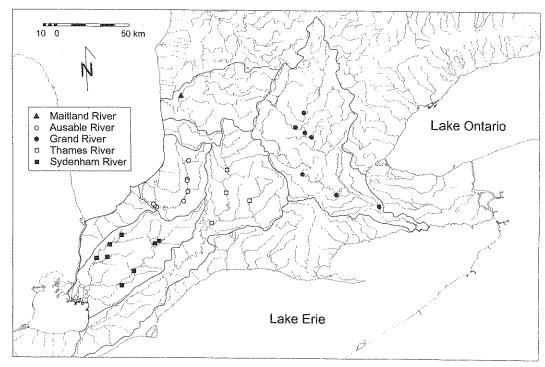


Fig. 1. Locations of sampling sites on rivers in southwestern Ontario, Canada.

surveys. Several of the sites we surveyed in 1997 had been surveyed 2 to 6 y earlier by Clarke (1992), Mackie (1996), or Morris (1996) using shorter search times, and in all cases our surveys yielded more live species and more individual mussels at a given site (Metcalfe-Smith et al. 1998). Although it was clear that longer search times were better than shorter search times for determining the status of a mussel community, the amount of sampling effort required for an adequate assessment remained unknown.

The objectives of this study were to determine the effect of increasing sampling effort in a timed search survey on 1) the detection of rare species, and 2) the number of species found at a given site or in a given river, i.e., the estimate of species richness. As the average sampling effort applied in other recent timed search surveys was ~ 1.5 p-h (see above), this effort was used as a point of reference.

Methods

The focus of our mussel surveys in southwestern Ontario rivers was to determine the occurrence and distributions of rare species, so we targeted sites that had supported rare species or diverse communities in the past. Thirty-seven sites were surveyed in 1997, and the results were used to select further sites for survey in 1998 (e.g., historical sites that had been missed, new sites in promising reaches). Twenty-eight sites on 5 rivers were surveyed between 4 August and 2 September 1998 (Fig. 1).

The riverbed at most sites was visually searched by a 3-person team using waders, polarized sunglasses, and Waterview underwater viewers for a period of 1.5 h or a total sampling effort of 4.5 p-h. Exceptions were several sites on the North Sydenham and lower East Sydenham rivers where visibility was very poor (maximum depth at which the streambed was clearly visible was generally <15 cm), necessitating searches by feel. Sites on the lower Ausable River were not as turbid (streambed generally visible at depths of ≤25 cm), but jagged, rocky substrates made using the Waterviews® difficult at times and tactile sampling was also used as required. The Grand, Thames, and Maitland rivers were clear (streambed generally visible to 1 m depth). At each site, the survey

period of 1.5 h was divided into 3 intervals of 30 min each. At the end of each interval, the 3 members of the survey team combined all the live specimens they had found into 1 mesh diver's bag, which was then labeled and left submerged in the river while the search resumed for the next interval. At the end of the survey period, all live mussels collected in each time interval were identified, counted, and returned to the river.

The main intent was to find as many species as possible, and to maximize our chances of finding certain rare species of interest. Therefore, our strategy was to search as many areas of suitable mussel habitat as possible, focusing especially on habitat types that had produced rare species or numerous species during previous surveys in these rivers (e.g., riffles/runs, margins of islands, around fallen trees). There was no fixed search pattern; rather, team members spread out in all directions in search of suitable habitat. After each of the first 2 sampling intervals, team members would go on to inspect additional habitats within the survey area, change direction and resurvey areas already covered by another team member, or continue examining a promising location. We neither searched entirely new reaches in succeeding time intervals, nor did we equally cover all portions of the survey area in each time interval.

To determine the effect of increasing sampling effort on the detection of rare species, it was 1st necessary to define the terms used. *Rare* species were those represented by 1–5 individuals 4.5 p-h⁻¹ site⁻¹, *uncommon* species were those with 6–10 individuals 4.5 p-h⁻¹ site⁻¹, and *common* species were those with >10 individuals 4.5 p-h⁻¹ site⁻¹. These definitions are similar to Strayer (1979). The terms refer to the number of individuals of a particular species found at a particular site, and not to the species' official conservation status rank. Thus, some species could fall into the rare category at 1 site, the uncommon category at another site, etc.

Results

Site characteristics

Time was the limiting factor in this study, so the area that could be surveyed at a particular site depended on stream width, water clarity, amount of suitable habitat available, and num-

TABLE 1. Numbers of 1st occurrences of rare, uncommon, and common species during each collection time interval.

Occurrence category	Time interval (person-hours)					
(no. individuals/ site)	1 st (1.5)	2 nd (3.0)	3 rd (4.5)	Totals and (%)		
Rare (1-5)	54	37	37	128 (55)		
Uncommon (6-10)	28	3	0	31 (13)		
Common (>10)	73	2	0	75 (32)		
Total occurrences	155	42	37	234		

bers of mussels present. The length of reach searched ranged from 55 to 575 m, but >80% of sites were 100 to 300 m long. Stream widths ranged from 4 to 275 m (median = 20 m); all sites were wadable. The numbers of mussels found varied from 5 to >1000. Maximum depth ranged from 0.7 to >1 m except for 2 sites that were a bit shallower, and the average depth searched ranged from 0.2 to 0.5 m.

Substrates in the Grand River consisted of a relatively even mixture of rubble, gravel, and sand; those in the Thames River were similar, but had proportionately more rubble and some boulder. The Maitland River site was 80% rubble. Substrates in the Ausable River were mostly gravel with substantial amounts of rubble and boulder, whereas those in the Sydenham River were mainly gravel and silt with $\sim \! 10\%$ each of boulder, sand, and muck.

Detection of rare species

We found 3540 mussels belonging to 28 species at the 28 survey sites. The total number of species occurrences, defined here as the presence of a species at a site, was 234. For each of these occurrences, we determined the time interval in which each rare, uncommon, and common species was 1st observed at each site. Fiftyfive percent of occurrences were for rare species, 13% were for uncommon species, and 32% were for common species (Table 1). Of the rare species occurrences, 54 of 128 (42%) were found during the first 1.5 p-h of searching, 29% were found in the second 1.5 p-h interval or with 3.0 p-h of effort, and 29% were found in the last 1.5 p-h, thus requiring 4.5 p-h of searching to be detected. Proportions for the uncommon species occurrences were 90%, 10%, and 0%, respective-

TABLE 2. Percentage of total species found in each time interval at each site on the Grand (GR), Thames (TR), Sydenham (SR), Ausable (AR), and Maitland (MR) rivers.

	Total -	Time interval			Total -	Time interval			
Site	species	1 st	2 nd	3rd	Site	species	Ist	2 nd	3rd
SR-16°	2	100	0	0	AR-1	8	75	0	25
4R-2	3	0	100	0	AR-3	9	89	11	0
SR-19	4	50	25	25	AR-4	9	22	0	78
GR-20	4	25	75	0	GR-22	9	67	11	22
GR-23	4	50	50	0	TR-14	9	44	44	12
FR-12	4	25	25	50	GR-21	10	90	0	10
R-13	4	75	0	25	AR-5	11	64	18	18
R-14ª	5	60	20	20	AR-6	12	75	8	17
R-15 ^a	5	100	0	0	AR-7	12	92	8	0
AR-1	6	50	0	50	AR-8	13	83	17	0
R-13ª	6	67	33	0	SR-11	13	54	38	8
GR-18	7	86	0	14	SR-10	14	29	50	21
GR-24	7	86	14	0	SR-12	16	75	6	19
R-15	7	71	14	15	SR-17	21	76	10	14
					Mean		63%	21%	16%

^a Sites on the North Sydenham River; all other Sydenham River sites were on the East Sydenham River.

ly, and those for the common species occurrences were 97%, 3%, and 0%, respectively. Thus, if we had surveyed for only 1.5 p-h, which is similar to the amount of effort commonly used in recent surveys, we would have detected at least 90% of the common and uncommon species at our survey sites, but <50% of the rare species. Only 35% of occurrences in the 1st time interval were for rare species (Table 1). The proportions of 1st time occurrences of rare species were much greater in the 2nd and 3rd time intervals; in fact, all new species found in the 3rd interval were rare.

The detection of rare and endangered species was our main concern, so it is of interest to know if we would have missed some occurrences of these species had we used less sampling effort. Data were examined for 3 target species, namely, the northern riffleshell (*Epioblasma torulosa rangiana*), rayed bean (*Villosa fabalis*), and wavy-rayed lampmussel (*Lampsilis fasciola*), which were recently listed as nationally endangered by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC 1999). There were a total of 10 occurrences of these species—3 in the 1st time period, 5 in the 2nd time period, and 2 in the 3rd time period.

Estimate of species richness

The % of all species found for the 1st time in each time interval was calculated for each site

(Table 2). These percentages varied considerably from site to site; however, an average of 63% of the species found at a given site were 1st observed in the first 1.5 p-h, 21% were 1st observed in the second 1.5 p-h, and 16% were discovered during the last 1.5 p-h. Thus, we could routinely expect to miss 37% of the species at a site by using only 1.5 p-h of sampling effort.

We compared the number of sites where each species was detected using 1.5, 3.0, or 4.5 p-h of sampling effort, with its catch rate. Catch rates on a per p-h basis were calculated using the total number of specimens found over all 28 sites by the end of the survey, i.e., after expending 4.5 p-h of sampling effort (Fig. 2). For species with catch rates ranging from 0.22 to 283/p-h over all sites, an increase in sampling effort nearly always resulted in an increase in the number of sites where they were found. Ptychobranchus fasciolaris, for example, had a catch rate of $\sim 9/p-h$ over all sites, and was found at 4 sites after 1.5 p-h of effort, 5 sites after 3.0 p-h, and 7 sites after 4.5 p-h. Villosa iris had a catch rate of $\sim 1/$ p-h and was found at 1, 3, and 4 sites after 1.5, 3.0, and 4.5 p-h effort, respectively. Each of these sites was on a different river, so V. iris would have been excluded from the species lists of 3 rivers if only 1.5 p-h of sampling effort had been used. Epioblasma triquetra had a capture rate of 0.22/p-h; it was found at only 1 site, and

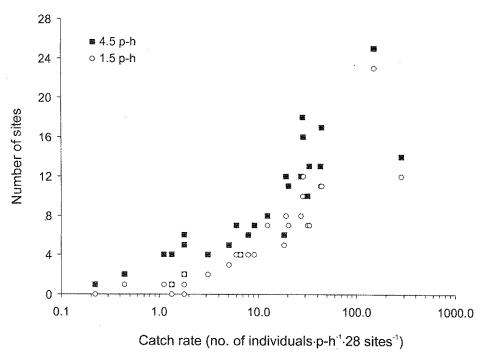


Fig. 2. Number of sites where each of the 28 species was detected during 1.5 and 4.5 person-hours (p-h) of sampling effort, as compared with the catch rate for that species. For clarity, only the data for the 1.5 and 4.5 p-h time periods are presented.

then only after 4.5 p-h of effort. At the other end of the spectrum, *Lasmigona costata* was the most widely distributed and 2nd most abundant species with a catch rate of about 150/p-h. It was found at 23, 24, and 25 of the 28 survey sites after 1.5, 3.0, and 4.5 p-h of sampling effort, respectively. Only 2 species were detected during the first 1.5 p-h of sampling at all sites where they were found, namely, *Quadrula pustulosa* (catch rate of 1.8/p-h) and *Truncilla truncata* (catch rate of 6.7/p-h).

TABLE 3. Species richness for each river after each sampling effort. The Maitland River was excluded because only 1 site was sampled.

River (no. of sites)	Sampling effort (person-hours)			
	1.5	3.0	4.5	
North Sydenham (4)	8	8	9	
Thames (4)	8	13	15	
Ausable (8)	15	17	18	
Grand (7)	16	17	18	
East Sydenham (4)	17	20	24	

Species richness also increased with increasing sampling effort for each of the studied rivers (Table 3). A sampling effort of 1.5 p-h was clearly insufficient for estimating overall species richness (i.e., obtaining a species list) for these rivers. In the North Sydenham River, which supported the fewest species, most species were detected within 3.0 p-h. In the Ausable and Grand rivers, an effort of 4.5 p-h appeared to be adequate. However, there was no indication that an asymptotic value had been reached by the end of the survey in either the Thames or East Sydenham rivers.

Discussion

Our study showed that an increase in sampling effort dramatically improved the detection of rare species of mussels during timed search surveys at sites on the Grand, Thames, Sydenham, Ausable, and Maitland rivers in southwestern Ontario. Fewer than 50% of the rare species were detected after 1.5 p-h of effort, which is similar to the sampling effort most often used in timed search surveys (e.g., Mackie

1996, Morris and Di Maio 1998, Obermever 1998). In fact, 70% of encounters with 3 federally endangered species, E. t. rangiana, V. fabalis, and L. fasciola, would have been missed if the search had ended after 1.5 p-h. Our findings also showed that most species occurrences were for species that would be considered rare (55% of all occurrences were for species represented by only 1-5 individuals at a site). Because rare species are such a significant component of the community, it is important to use a sampling effort that is appropriate for detecting them. Even the species that were common in general were rare at some sites, so their full distributions would not be known without sufficient sampling effort.

Surveys that use the timed search technique to assess mussel populations are relatively less expensive and time-consuming than quadrat surveys (Strayer et al. 1997). Our qualitative sampling effort of 4.5 p-h/site was more intensive than most, nearly matching the effort expended by Strayer et al. (1997) during quadrat sampling at 53 sites on wadable streams between New Hampshire and North Carolina (average effort = 5.2 p-h/site). This level of effort may seem excessive for some purposes, but how much effort is needed to arrive at a reliable estimate of species richness using qualitative methods? Miller and Payne (1993) and Hornbach and Deneka (1996), conducting qualitative surveys on large rivers using scuba divers, found that they needed to collect between 100 and 700 individuals per site to properly characterize the mussel assemblage. Hornbach and Deneka (1996) suggested that differences in the patchiness of the substrate and the mussel community may account for differences in the amount of effort required at a particular site.

We were interested in both obtaining a species list for each river, and a species list for each site to compare with historical data that were generally of a presence/absence nature. We found that 1.5 p-h of effort was not enough for estimating the number of species present in a river, and that 4.5 p-h was probably adequate for some, but not all, rivers. We speculate that rivers with many rare species, low densities, and/or considerable site-to-site variation in community composition, will require more sampling effort. New species were still being found during the last time interval at 18 of our 28 sites (see Table 2), suggesting that our estimates of species rich-

ness may be low for most sites. At a site on the Sydenham River for which good historical data were available (site SR-12, Table 2), only 16 of 24 potential species had been found by the end of our 4.5 p-h survey. We searched this site for an additional 1.5 p-h, and found 2 more species. Other researchers working at this site on the same day found another 2 species using 6.0 ph of effort (D. Zanatta, University of Guelph, unpublished data). In 1999, a further 2 species were found during 11.0 p-h of quadrat sampling (78 1 m² quadrats × 8.2 person-min/quadrat) and another was found just outside the sampling area (J. L. Metcalfe-Smith and J. Di Maio, unpublished data). All sampling took place within the same 120 m × 30 m reach. Thus, it took 23 p-h of sampling effort to detect 23 of the 24 species expected to occur at this site.

If the objective of a survey is to arrive at a species list for a river, and the amount of time available to conduct the survey is fixed, then the question is how best to allocate the sampling effort among the sites. There is an inherent trade-off whereby the more time spent at a site, the fewer sites that can be visited. Strayer (1999) suggested that increasing the number of sites may be a better approach to detecting declines in mussel populations (in terms of density or number of sites occupied) than increasing the sampling effort/site. Comparisons between our data and those from other recent surveys on the Grand and Thames rivers would lead us to recommend the opposite. Morris (1996) surveyed 30 sites on the Thames River using a sampling effort of 1 p-h/site and found 18 live species. We surveyed 16 sites on this river from 1997 to 1998 (Metcalfe-Smith et al. 1998; this study) using a sampling effort of 4.5 p-h/site, and detected 23 live species. Note, however, that our total sampling effort was about twice that of Morris's. Mackie (1996) surveyed 70 sites on the Grand River in 1995 using a sampling effort of 1.5 p-h/site for a total effort of 105 p-h, and detected 18 live species. We surveyed 24 sites on this river from 1997 to 1998 using a sampling effort of 4.5 p-h/site for a total effort almost identical to Mackie's (108 p-h), and found 25 live species. All live species found by Morris (1996) and Mackie (1996) were also found by us.

One of the advantages of qualitative sampling is that many different habitats can be explored relatively quickly (Kovalak et al. 1986). We found that spending additional time at a site al-

lowed us to become more familiar with the various types of habitat that mussels occupy and, thus, to locate additional productive areas that might otherwise have been overlooked. We also learned much about the habitat preferences of individual species during nearly 300 p-h in the field in 1997 and 1998.

Our results illustrate what is almost intuitive: more species will be detected with more sampling effort. However, sampling time becomes critical when dealing with rare species; in our study, most of the rare species were found during the 2nd and 3nd time intervals, i.e., using 3.0–4.5 p-h of sampling effort. Researchers who rely on timed search surveys to determine the occurrence of rare and endangered species must carefully consider the amount of time they spend at their study sites. If researchers do not use sampling techniques and efforts that are capable of detecting these species, a significant amount of information about the mussel community will be lost.

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Literature Cited

- CLARKE, A. H. 1992. Ontario's Sydenham River, an important refugium for native freshwater mussels against competition from the zebra mussel *Dreissena polymorpha*. Malacology Data Net 3:43–55.
- COSEWIC (Committee on the Status of Endangered Wildlife in Canada). 1999. Canadian species at risk, April 1999. COSEWIC, Environment Canada, Ottawa, Ontario. (Available from: COSEWIC Secretariat, Canadian Wildlife Service, Environ-

- ment Canada, Ottawa, Ontario K1A 0H3 Canada.)
- Downing, J. A., and W. L. Downing. 1992. Spatial aggregation, precision, and power in surveys of freshwater mussel populations. Canadian Journal of Fisheries and Aquatic Sciences 49:985–991.
- GREEN, R. H., AND R. C. YOUNG. 1993. Sampling to detect rare species. Ecological Applications 3:351– 356.
- HORNBACH, D. J., AND T. DENEKA. 1996. A comparison of a qualitative and a quantitative collection method for examining freshwater mussel assemblages. Journal of the North American Benthological Society 15:587–596.
- KOVALAK, W. P., S. D. DENNIS, AND J. M. BATES. 1986. Sampling effort required to find rare species of freshwater mussels. Pages 34–45 in B. G. Isom (editor). Rationale for sampling and interpretation of ecological data in the assessment of freshwater ecosystems. Special Technical Publication 894. American Society for Testing and Materials, Philadelphia, Pennsylvania.
- MACKIE, G. L. 1996. Diversity and status of Unionidae (Bivalvia) in the Grand River, a tributary of Lake Erie, and its drainage basin. Report prepared for Lands and Natural Heritage Branch, Ontario Ministry of Natural Resources, Peterborough, Ontario. (Available from: Lands and Natural Heritage Branch, Ontario Ministry of Natural Resources, Box 7000, Peterborough, Ontario K9J 8M5 Canada.)
- METCALFE-SMITH, J. L., S. K. STATON, G. L. MACKIE, AND I. M. SCOTT. 1999. Range, population stability and environmental requirements of rare species of freshwater mussels in southern Ontario. NWRI Contribution No. 99-058. National Water Research Institute, Environment Canada, Burlington, Ontario.
- METCALFE-SMITH, J. L., S. K. STATON, G. L. MACKIE, AND E. L. WEST. 1998. Assessment of the current conservation status of rare species of freshwater mussels in southern Ontario. NWRI Contribution No. 98-019. National Water Research Institute, Environment Canada, Burlington, Ontario.
- MILLER, A. C., AND B. S. PAYNE. 1993. Qualitative versus quantitative sampling to evaluate population and community characteristics at a large-river mussel bed. American Midland Naturalist 130: 133–145.
- MORRIS, T. J. 1996. The unionid fauna of the Thames River drainage, southwestern Ontario. Report prepared for the Lands and Natural Heritage Branch, Ontario Ministry of Natural Resources, Peterborough, Ontario. (Available from: Lands and Natural Heritage Branch, Ontario Ministry of Natural Resources, Box 7000, Peterborough, Ontario K9J 8M5 Canada.)
- Morris, T. J., and J. Di Maio. 1998. Current distri-

- butions of freshwater mussels (Bivalvia: Unionidae) in rivers of southwestern Ontario. Malacological Review 31:9–17.
- OBERMEYER, B. K. 1998. A comparison of quadrats versus timed snorkel searches for assessing freshwater mussels. American Midland Naturalist 139: 331–339.
- STRAYER, D. L. 1979. Some recent collections of mussels from southeastern Michigan. Malacological Review 12:93–95.
- STRAYER, D. L. 1999. Statistical power of presence-absence data to detect population declines. Conservation Biology 13:1034–1038.
- STRAYER, D. L., S. CLAYPOOL, AND S. J. SPRAGUE. 1997. Assessing unionid populations with quadrats and timed searches. Pages 163–169 *in* K. S. Cummings, A. C. Buchanan, C. A. Mayer, and T. J. Naimo (editors). Conservation and management

- of freshwater mussels II: initiatives for the future. Proceedings of an Upper Mississippi River Conservation Committee symposium, 16–18 October 1995, St. Louis, Missouri. UMRCC, Rock Island, Illinois.
- VAUGHN, C. C., C. M. TAYLOR, AND K. J. EBERHARD.
 1997. A comparison of the effectiveness of timed searches vs. quadrat sampling in mussel surveys.
 Pages 158–162 in K. S. Cummings, A. C. Buchanan, C. A. Mayer, and T. J. Naimo (editors). Conservation and management of freshwater mussels II: initiatives for the future. Proceedings of an Upper Mississippi River Conservation Committee symposium, 16–18 October 1995, St. Louis, Missouri. UMRCC, Rock Island, Illinois.

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