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Mantle biopsy: a technique for nondestructive tissue-sampling of freshwater mussels

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Abstract. Mantle biopsy is a means of obtaining tissue samples for genetic, physiological, and contaminant studies of bivalves; but the effects of this biopsy on survival have not been determined. We describe a simple technique for obtaining such samples from unionacean bivalves and how we compared survival among biopsied and control organisms in field experiments. Survival was not significantly different between treatment and control groups. Power estimates for these results were between 0.42 and 0.73. Results were similar among species and among habitats. Mantle biopsy is a technique that allows genetic, biochemical, and contaminant studies of mussel populations when destruction of individuals should be avoided.

Key words: Unionidae, nondestructive sampling, freshwater mussels, endangered species, mantle biopsy, *Actitwnais, Quadrula.*

Freshwater mussels of the families Unionidae and Margaritiferidae are a conspicuous element of benthic communities in North America. Human activities, however, including impoundment and channelization of rivers, commercial shell harvesting, water pollution, and introduction of non-native species, have caused dramatic declines in abundance and diversity of this fauna (Neves 1993, Williams et al. 1993). Approximately 72% of native freshwater mussel taxa are considered endangered, threatened, or of special concern. Consequently, government agencies and private environmental organizations have made conservation of the North American mussel fauna a priority (Stolzenburg 1992, Williams et al. 1993).

Conservation requires information on genetic structure of populations (Sandlund et al. 1992) and fitness of individuals. Traditionally, techniques used to collect tissue samples for genetic analyses (Stiven and Alderman 1992), biochemical measures of fitness (e.g., glycogen and lipid concentrations, enzyme activity (Haag et al. 1993)), and contaminant burdens (Tessier et al. 1984) in mussels have required the death of study animals. This requirement has denied such techniques for studies in which animals should not be killed, such as those involving 1) threatened and endangered species or otherwise sensitive populations, or 2) repeated sampling from study animals. Nondestructive tissue-sampling techniques are needed so that studies of genetic structure and fitness of mussel populations are not hindered by requiring the death of study animals.

Mantle biopsy has been used to obtain tissue samples for genetic studies of rare species in the unionid genus *Lampsilis* (Stiven and Alderman 1992) and the giant marine clam *Tridacna maxima* (Benzie and Williams 1992), but the effects of this technique on survival were not reported in either study. Before such a technique can be routinely employed, it is imperative to determine whether mantle biopsies increase the mortality of subjects.

We developed a method for obtaining tissue samples by mantle biopsy and we estimated mortality associated with this technique using two nonendangered species of unionids from two different habitats, a reservoir and a small river. The results of these studies were used to determine if mantle biopsy increases the mortality of subjects and whether the technique may be safely used to obtain tissue samples for genetic, biochemical, and contaminant studies.

Methods

Our biopsy method consisted of gently forcing the shell open to a gape of approximately 10 mm, inserting a wooden wedge to hold the

shell open, and clipping a 1-cm² piece of mantle tissue with forceps and fine scissors. The sample was removed from the extreme ventral margin of the mantle at a position approximately ⅓ of the total length from the anterior end. This biopsy location was chosen to avoid damaging the posterior region of the mantle which is modified to form siphonal apertures and papillae or other sensory or reproductive structures (McMahon 1991). Biopsies were performed in the field, without using sterile techniques. The tissue samples obtained were approximately 34.3 mg (±2.3) (SE), large enough to resolve a minimum of 14 allozyme loci per individual using cellulose acetate and starch gel electrophoresis.

We tested this method using the most common mussel species at two field sites. *Quadrula quadrula* was used at a site in an embayment of Kentucky Lake at Tennessee River mile 44.5, Graves Co., Kentucky, near Hunter Hancock Biological Field Station of Murray State University. Kentucky Lake is an artificial impoundment of the Tennessee River, and embayments are characterized by depths of 3-10 m, slow to non-existent currents, and substrates of silt or mud. *Actinonaias ligamentina* was used at a site on the Licking River at Moore's Ferry, Bath Co., Kentucky, where the river is approximately 60 m wide, with shallow riffles (<0.5 m deep), swift currents, and sand and gravel substrates.

At each site, animals were collected by SCUBA diving (186 *Quadrula quadrula,* June 1993) or wading *(206 Actinonaias ligamentina,* May 1993), and randomly assigned to either a treatment or a control group. Treatment individuals were marked with a single groove filed on the right valve; controls were marked with two grooves on the left valve. A tissue sample was removed from all treatment individuals, then both groups were returned to the water. Marking and biopsying of individuals was done at the field sites, within 3 h of collection. In Kentucky Lake, animals were placed in four 1.4 **X** 0.8-m wire enclosures at a depth of 3.5 m. Approximately 50 individuals were placed in each enclosure without regard to their group assignment. Enclosures were used to facilitate recovery of the animals at the end of the experiment and to exclude muskrats, which potentially can harvest large numbers of mussels (Neves and Odom 1989). In the Licking River, enclosures were not used because high discharge during winter and spring likely would have destroyed the enclosures, and an absence of shell middens indicated little predation by muskrats. To facilitate recovery of animals at this site, we placed all individuals in a single "patch" approximately 5 X 5 m, which we relocated by mapping this section of stream. Total sample sizes consisted of 92 treatment and 94 control animals for Q. *quadrula* and 102 treatment and 104 control animals for *A. ligamentina.*

The field sites were censused in late September *(Q. quadrula)* and early October 1993 *(A. ligamentina)* to assess survival during the summer, and again in June *(A. ligamentina)* and July 1994 *(Q. quadrula)* to assess survival after one year. We used a one-tailed z-test of difference between proportions (Devore and Peck 1986) to examine the null hypothesis that proportion of individuals recovered alive (no. recovered alive/no. marked) did not differ between treatment and control groups at each site. The alternative hypothesis was that proportion of individuals recovered alive was lower for the treatment group. Because these tests failed to reject the null hypothesis, we performed a power analysis to determine the probability of committing a Type II error (Zar 1984). Fisher's Exact Test was used to compare results between species and habitats because initial ratios of control: treatment were slightly different for the two experiments.

Results and Discussion

We recovered shells of all living and dead *Quadrula quadrula* placed in the enclosures, both after 3.5 mo and after 13 mo (Table 1). We recovered 50% and *54%* of *Actinonais ligamentina* shells placed in the river patch after 3 and 12 months, respectively (Table 1). Recovery was lower in the river habitat due to high winter discharge, which may have washed some individuals downstream, and our inability to use enclosures in this environment.

We found no significant differences in proportion of animals recovered alive between treatment and control groups for either species on either sample date (Table 1). For *Q. quadrula,* upper limits of 95% confidence intervals for the difference in proportion alive between control and treatment were 0.0950 after 3.5 mo and 0.0556 after 13 mo. However, lower limits extended below zero (Fig. 1). For *A. ligamentina,* upper limits of 95% confidence intervals were

TABLE 1. Number (proportion) of mussels recovered alive after 3-3.5 and 12–13 mo following mantle biopsies (for treatment group). n = number of mussels in each group at the beginning of the experiments; 100% of the Quadrula *quadrula* were recovered after both time intervals, while 50% and *54%* of *Acfinonais ligamentina* were recovered after 3.5 and 13 mo, respectively. The z-scores are results of l-tailed tests for difference of proportions between control and treatment for each sample date.

	n	3[a]–3.5[b] mo	12[a]–13[b] mo
Quadrula quadrula (Kentucky Lake)			
Control	94	86 (0.915)	79 (0.840)
Treatment	92	82 (0.891)	80 (0.870)
z-score (p)		0.54 (0.29)	-0.57 (0.28)
Acfinonais ligamentina (Licking River)			
Control	104	56 (0.539)	58 (0.558)
Treatment	102	47 (0.461)	53 (0.520)
z-score		1.12 (0.13)	0.34 (0.36)

[a] *Acfinonais ligamentina*
[b] *Quadrula quadrula.*

higher (0.1920 and 0.1523 for 3 mo and 12 mo, respectively), because the proportions of control and treated individuals recovered were both near 50% and these values result in a maximum standard deviation for binomial distributions (Zar 1984). However, lower limits for these intervals also extended below zero (Fig. 1). Power was calculated assuming that the treatment caused a true reduction of 0.1 in the proportion of individuals recovered alive, with $\alpha = 0.05$. For *Q. quadrula,* power = 0.73 after 3.5 mo and 0.60 after 13 mo. For *A. ligamentina,* power = 0.42 after 3 mo and after 12 mo. We feel that a reduction in proportion recovered alive of 0.1 represents a minimal effect. Power of the experiments would be considerably greater if we defined an effect as occurring only with a greater difference between control and treatment (say, 0.15 or 0.2). The difference in proportion of control and treatment animals recovered after termination of the experiment did not differ significantly between species (Fisher's Exact Test, Fisher statistic = 0.1765, $p = 0.6744$).

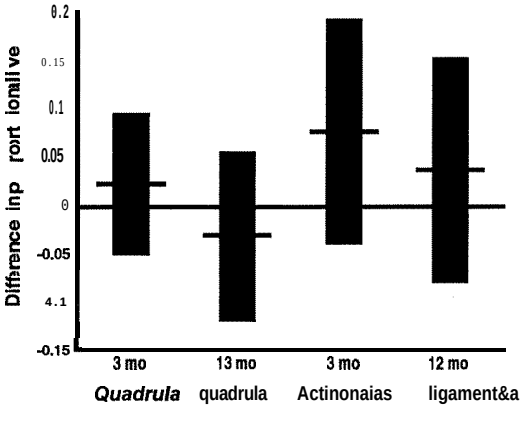

FIG. 1. 95% confidence intervals for the difference in proportion alive between treatment (receiving mantle biopsies) and control (no biopsy) groups for *Quadrula quadrula* (Kentucky Lake) and *Actinonaias ligamentina* (Licking River) after 3-3.5 and 12-13 mo.

We were unable to detect an increase in mortality in individuals subjected to mantle biopsies. The two species used in this study represent two subfamilies of freshwater mussels (*Actinonais ligamentina*—Lampsilinae; *Quadrula quadrula*—Ambleminae) that differ widely in ecological and reproductive characteristics (Clarke 1981). Although we were unable to evaluate the effects of habitat variation on species-specific survival, results of experiments were similar for these two species and two different habitats. Sublethal effects were not quantified in this study. However, when recovered, all control and treatment mussels were completely buried and naturally oriented in the substrate, were siphoning, and were responsive to stimuli. No evidence of disease or internal damage was observed. Stressed mussels are often found lying on top of the substrate, may appear moribund, and are unresponsive to physical stimulation (Bills et al. 1992, Miller et al. 1984). Shell abnor-

malities attributed to natural injury of the shell-secreting portion of the mantle margin are commonly seen in freshwater mussels (Coker et al. 1921). Evidence of these injuries is often seen as a growth check or deformity at some earlier point on the shell, showing that such injuries are not lethal and probably minimally affect fitness. We conclude that there is no evidence to show that mantle biopsies result in increased mortality in mussels, or that responses to the technique may differ among species or habitats.

This technique will allow researchers to investigate the ecological genetics and physiological ecology of freshwater mussel populations even when study animals must remain alive. We have used this technique to study genetic structure of mussel populations from several locations (Berg and Guttman, unpublished data). Recent work focusing on the use of biochemical indicators of fitness such as glycogen content and cellulase activity in freshwater mussels (Farris et al. 1988, Haag et al. 1993) and on the use of mussels as biomonitors (Tessier et al. 1984) has relied on destructive sampling to obtam tissues. However, the use of mantle biopsies may make it possible to routinely employ these biochemical assays with a wider variety of mussel species and more flexible study designs. Because the mantle is a site of metal accumulation in freshwater mussels (Tessier et al. 1984), the biopsy technique may be especially useful for the monitoring of metal contamination in unionid populations. As the decline in mussel abundance and diversity continues, researchers and management agencies will want to know more about the ecology and population genetics of these organisms. Nondestructive techniques such as mantle biopsy will allow such work to proceed with minimal damage to sensitive populations.

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