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K'titel rechts: Sampling of freshwater mussels K'titel links: Terry D. Richardson and Paul Yokley Jr.

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# A note on sampling technique and evidence of recruitment in freshwater mussels (Unionidae)

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Abstract: Total substratum removal with subsequent sieving is the best means for assessing successful recruitment events in freshwater bivalve populations. We sampled three previously surveyed sites using total substratum removal and found evidence for recent recruitment at one of the three sites. This evidence had been previously overlooked when only diver searches were used. This study demonstrates that unequivocal evidence of recruitment can be found with minimal sampling effort and indicates the need for total substratum removal sampling in freshwater bivalve surveys to accurately assess recruitment.

#### Introduction

Recruitment of juveniles into animal populations reflects successful reproduction by adults and is one of the best means to assess population viability. This is especially true for animals like freshwater mussels (Bivalvia: Unionidae) that have larval stages whose mortality is affected by factors that do not necessarily affect adults (larval predators, finding a suitable host, differential susceptibility to adverse environmental factors, etc.) (MCMAHON 1991).

Freshwater mussels have complex life cycles involving parasitic larvae (glochidia) that depend upon a host fish to complete development (Coker et al. 1921, McMahon 1991). The eggs are fertilized internally and develop in the marsupial gills until their release as glochidia. Hence, gills of females are often examined for the presence of developing glochidia as a means of determining individual reproduction (e.g., Haggerry et al. 1995). However, population growth or sustained abundance also depends upon successful recruitment. Examining reproductive activity in adults only determines if adults are capable of producing offspring and provides no information about recruitment.

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Additional information on the presence of small size classes is necessary to give a complete description of population demographics and reproductive success (sensu MILLER & PAYNE 1988).

Recent efforts (BUTLER 1993) have indicated that two uncommon endemic species of unionids in the Apalachicola Region, the purple bankclimber, *Elliptoideus sloatianus* (proposed to be listed as threatened), and the fat three-ridge, *Amblema neisleri* (proposed to be listed as endangered), have populations with unknown viability suggesting that the mussels may not be reproducing or at least are not experiencing successful recruitment. These conclusions, however, were based upon gross examination of female gills, and search and feel methods for collecting mussels. Usually, search and feel methods are biased against finding recent recruits (see MILLER & PAYNE 1988 for a discussion on sampling bivalve populations). The goal of our study was to examine total substratum samples from three previously surveyed sites to determine if populations of either the purple bankclimber or fat three-ridge show evidence of recent recruitment that may have been overlooked using diver searches.

#### Methods

Three sites along the Apalachicola River, U.S.A., were chosen based on 1991–92 field records and data from the U.S. Fish and Wildlife Service and National Biological Survey. The only criterion used in choosing sites was the reported presence of either the purple bankclimber or the fat three-ridge. The first site was located approximately 0.6 miles downstream from J. Woodruff Dam in Gadsden County, Florida at navigation mile 105.2 along Race Bluff at Race Shoals (30° 40′ N, 84° 54′ W). The second site was downstream at navigation mile 92.5 just below Rock Bluff Landing in Liberty County, Florida (30° 30′ N, 85° 01′ W). The final site was located at navigation mile 21.8 (29° 59′ N, 85° 01′ W) just upstream from Brickyard Island in Franklin County, Florida.

Samples were taken using methods similar to MILLER & PAYNE (1988). Briefly, at each site six samples were taken by experienced divers in areas where adult mussels were observed. A metal quadrat (0.25 m²) was placed on the substratum and the material within excavated until one of the following criteria was met: (1) a depth of at least 15 cm was reached throughout the quadrat; (2) solid bedrock substratum was encountered in which case all the overlying substratum was removed; (3) solidly compacted clay was encountered in which case the substratum was removed down to and including the top 3-4 cm of the compacted clay. All substratum material was scooped into a bucket, returned to the boat, and washed over a 6 mm sieve. Unionids were removed from the material retained on the sieve, identified (using HEARD 1979), and photographed. All but a representative sample of the mussels were returned to the river bottom and placed in their original orientation. The reserved specimens were submitted to the Ohio State University's Museum of Biological Diversity in Columbus, Ohio, U.S.A. and have been verified and retained in the museum's collection (OSUM: 1995: 0052 and 0053).

#### Results

Several mussel species were found at the site below J. Woodruff Dam (Table 1). Elliptio crassidens incrassata was the most common species sampled and had a relatively narrow range of shell sizes. Elliptoideus sloatianus was the next most common mussel sampled. All E. sloatianus shells at this site were highly eroded, old individuals and varied little in length. One relatively small Elliptio arctata (7.6 cm) was taken, but no young of any other species was encountered at this site. All specimens were found in sand and gravel in pits among the limestone bedrock.

At Rock Bluff Landing, only a single unionid was observed and none were taken in samples (Table 1). The site consists of a limestone bluff with a series of shelves leading to the deeper channel. Sand and gravel in pits among the limestone bedrock shelves and in the channel at the bottom of the bluff provided suitable unionid habitat and were where mussels had been taken previously (Butler 1993). Except for *Corbicula fluminea*, the entire area was mostly depauperate of bivalves.

Four species were found at the Brickyard Island site (Table 1). Glebula rotundata was the most common species at this site with Amblema neisleri the next most common. A wide size range including several small individuals was found for both G. rotundata (4 shells  $\leq 50$  mm) and A. neisleri (3 shells  $\leq 50$  mm) with all shells  $\leq 50$  mm shell length found in excavated samples. In

Table 1. Relative abundances at each site and the range of shell lengths of the bivalve species found at each of three sites on the Apalachicola River.

Site	Species	% Abundance per site <sup>a</sup>	Shell Length (mm) range
J. Woodruff			
Dam	Elliptio crassidens incrassata	59	91-99
	E. arctata	4	76
	Elliptoideus sloatianus	37	133-163
	Megalonaias boykiniana	n.a. <sup>b</sup>	n.a. <sup>b</sup>
	Lampsilis teres floridensis	n.a. <sup>b</sup>	n.a. <sup>b</sup>
Rock Bluff Landing <sup>c</sup> Brickyard	E. crassidens incrassata	n.a. <sup>b</sup>	
Island	Amblema neisleri	25	34-64
	M. boykiniana	4	135
	Glebula rotundata	65	31-64
	E. crassidens incrassata	4	87

<sup>&</sup>lt;sup>a</sup> Total number of unioinids collected = 49.

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<sup>&</sup>lt;sup>b</sup> These species were observed at the site but did not appear in samples.

<sup>&</sup>lt;sup>c</sup> No unionids were taken in any of the six samples.

addition, smaller shells of A. neisleri demonstrated a rather uniform size distribution (one individual from each of the following size classes: 2-3, 3-4, 4-5, and 5-6 pm). The smallest individuals of these two species had fewer than five growth rings (presumed to be annual growth rings). The smallest A. neisleri (2.4 cm shell length) was an apparently fresh dead shell with the hinge ligament still in place and which showed little sign of shell dissolution or periostracum decay. This specimen had 2-3 growth rings.

#### Discussion

Our findings indicate Amblema neisleri and Glebula rotundata have experienced relatively recent recruitment at the Brickyard Island site. The youngest shell found for A. neisleri suggests recruitment occurred possibly no longer than 3-4 years ago (based on growth rings, see Neves & Moyer 1988 for aging freshwater mussels) with some other individuals estimated to be 6-8 years old. Furthermore, the rather continuous size series for the fat three-ridge indicates that successful recruitment has been ongoing for several generations. Such continual recruitment implies that host fish and other factors necessary for successful reproduction and recruitment were present at the site at least up until 3-4 years ago.

The most recent recruitment event seems to have occurred as much as 3-4 years ago. The absence of a 1-3 year old group in the population could result from these individuals having been overlooked by relatively low sample numbers (e.g., nine shells for A. neisleri), but such a gap in recruitment is not uncommon for unionids. Many mussel populations where conditions are adequate for reproduction often have periods lacking recruitment (BAUER 1983, PAYNE & MILLER 1989). The adults may be reproducing each year, but, for whatever reason, the larvae do not survive to add to the population. As a result, successful recruitment into the adult population is sporadic depending on annual variation in larval survivorship and other factors.

When BUTLER (1993) surveyed this area, no females with glochidia were found. In addition, searches by divers found no evidence of successful recruitment of A. neisleri. Using search and feel methods, divers visually examine the river bottom for bivalves or use their hands to feel for mussels if visibility is limited. Unfortunately, recent recruits and juvenile bivalves are difficult to see and often burrow several centimeters into the sediment. As a result, juveniles can be missed when only searching and feeling are used. Recruitment of A. neisleri apparently occurred 3-4 and 6-8 years ago; small mussels reflecting these recruitment events would have been present at the time the BUTLER (1993) survey was conducted. This suggests juveniles may have been overlooked and that additional sampling using substratum removal accompa-



nied by sieving was needed to provide evidence of recruitment. In an earlier study, MILLER & PAYNE (1988) documented that total substratum removal techniques combined with sieving were necessary to assess accurately the size structure and demographics of unionid populations.

Although no young of other species were found at other sites, this may reflect the small number of individuals sampled rather than lack of recruitment. From the entire Apalachicola River only three sites were chosen and only six samples were taken at each site. However, MILLER & PAYNE (1988) point out that under some conditions as few as seven  $0.25\,\mathrm{m}^2$  quadrat samples are necessary for reliable estimates of unionid population density. Because densities in the Apalachicola River are less than those cited by MILLER & PAYNE (1988), it is likely that more than seven samples would be needed to provide a high probability of finding juveniles of relatively rare individuals like Amblema neisleri and Elliptoideus sloatianus. Because evidence of recruitment was found for at least one species, it argues unequivocally that recent recruitment has taken place and more substratum removal samples would likely find additional examples of recruitment.

In conclusion, using total substratum removal samples with sieving for assessing juvenile presence we found juveniles for two species at one of three sites. We believe this indicates that at least until 3-4 years ago conditions were favorable for unionid reproduction and viable populations existed. Our results reiterate those of MILLER & PAYNE (1988) suggesting that when examining demographic parameters like recruitment, it is important that bivalve populations be sampled using excavation and sieving techniques; relying solely on diver searches may lead to inaccurate assessments of population size structure.

#### **Acknowledgements**

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## BALCH & BINGHAM

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April 10, 1996

Mr. Sean C. Skaggs United States Department of the Interior Office of the Solicitor, Southeast Region Richard B. Russell Federal Building 75 Spring Street, S.W., Suite 304 Atlanta, Georgia 30303

> Re: Proposal to List Seven Freshwater Mussels as Endangered or Threatened ("Proposal"), 59 Fed. Reg. 39524 (Aug. 3, 1994).

Dear Mr. Skaggs:

In a telephone conversation with you in October of last year and in several subsequent conversations, I informed you that the Tri States Mussels Coalition had commissioned an independent scientific study related to the above-referenced proposal. Although we have known for some time that the scientific article describing that study had been peer reviewed and accepted for publication, we only recently received a copy of the "galley proof" for the article. I have enclosed a copy of the article for your use and distribution.

As you may recall, the Service claimed in its proposal that the seven proposed mussels were not reproducing, and based this critical conclusion on the Service's failure to find juvenile specimens of the proposed mussels during their mussel survey efforts in 1991. The Coalition and its scientists were extremely critical of the Service's "search and feel" sampling methods, but Service representatives did not believe the Coalition's criticisms were valid. Consequently, the Coalition funded its own scientific research, the results of which conclusively demonstrate that the Service's conclusions were not based on sound science. Although Coalition scientists only conducted "excavation and sieve sampling" at three sites on one river, they nevertheless found several juvenile specimens of one of the two proposed mussels known to inhabit that river. Considered in light of the Coalition's finding that these seven mussels were never abundant historically, these results call into question the Service's assertion that the mussels and other aquatic species in the Apalachicolan basin are in peril. We believe the Service should reconsider its proposal and many of the conclusions contained therein in light of this new scientific information.

### BALCH & BINGHAM

Mr. Sean C. Skaggs April 10, 1996 Page 2

When the Coalition met with you and Service representatives last Spring, you indicated that the Service would take any new scientific information into consideration if that information is available prior to the Service's final decision on this proposal. Although the Congressional moratorium on final listings is still in effect, we wanted to provide you and the Service with a copy of this article so that the Service would have time to consider it prior to making any decision. We will provide you with an "as published" copy of this article when it appears in print.

I trust that this letter and the enclosed article are helpful. Although I have attempted to provide a copy of the article to all appropriate Service personnel, I would appreciate your forwarding it on to any other Department of the Interior or Service employees who you believe should receive a copy. Please feel free to contact me at the above number if you have any questions.

Sincerely,

Glenn G. Waddell

GGW/rr enclosure

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cc:

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