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REGULAR ARTICLE

MUSSEL SPECIES RICHNESS ESTIMATION AND RAREFACTION IN CHOCTAWHATCHEE RIVER WATERSHED STREAMS

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ABSTRACT

We determined the number of samples necessary to accurately estimate species richness at three sites in the Choctawhatchee River watershed in Alabama and Florida. We sampled each site eight times using 5 person-hr timed searches with a combination of visual and tactile searching from June to October 2012. We estimated total species richness at each site using the Chao 2 estimator to construct rarefaction curves. We used these relationships to determine sampling effort necessary to detect 80%, 90%, 95%, and 99% of the estimated total species richness and the percentage of species detected with successive samples. We conducted the same analyses using a subset of the data including only federally threatened or endangered (TE) species. Species detection and effort requirements differed among streams and were primarily influenced by mussel abundance. We detected 62–88% of estimated total species richness with one sample, and detection of 90–99% of species required 2.1–8.0 samples. At two sites with high mussel abundance, detection of $\geq$90% of estimated total species richness required 1.3–2.2 samples, but five samples were required to detect a similar percentage of species at a site with lower mussel abundance. A single sample was sufficient to detect all TE species present at two sites where these species were abundant, but a single sample in a stream with lower mussel abundance detected only 45% of TE species, and eight samples were required to detect 90% of TE species.

Key Words: number of samples, species richness, freshwater mussels, endangered mussels, mussel assemblages

INTRODUCTION

Substantial declines in freshwater mussel populations in North America have occurred over the past several decades (Strayer et al. 2004; Shea et al. 2013; Haag and Williams 2014). Species richness estimation is an important component of biodiversity studies and conservation, especially when considering at-risk fauna (Boulinier et al. 1998; Kéry et al. 2009). Observations of trends in species richness can focus conservation efforts in areas where diversity is declining, since few studies show significant correlations between specific habitat variables and mussel assemblages (Strayer and Ralley 1993; Niraula et al. 2015a, 2015b). Determining true species richness at a site is seldom possible (Colwell and Coddington 1994); rather, richness typically is estimated from sample data, resulting in an underestimation of species richness, the extent of which is dependent on sampling effort (Hellman and Fowler 1999). The effort required to detect a reasonable percentage of species at a site is important to know when designing sampling programs.

Due to their clustered distribution and benthic nature, mussels are difficult to sample adequately, and species richness often is underestimated due to incomplete detection (false absences) (Strayer and Smith 2003; Shea et al. 2013; Wisniewski et al. 2013). Qualitative protocols have not been well tested with regard to species detection within a given reach (Huang et al. 2011). Recent studies have used occupancy

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modeling to explicitly quantify probability of nondetection (e.g., Meador et al. 2011; Wisniewski et al. 2013). This approach provides more accurate information on species richness and other community and population variables than can be obtained from most standard sampling methods, but occupancy modeling can be labor intensive and requires specific study designs.

Rarefaction and species accumulation curves provide an alternative to occupancy modeling that can be applied more easily and quickly to standard qualitative sampling methods. A species accumulation curve is constructed by plotting the cumulative number of species found at a site versus a measure of sampling effort (e.g., number of samples, person-hours) (Colwell et al. 2004). Sampling variability (e.g., environmental factors and human bias) affects the shape of a species accumulation curve such that different sampling events provide different curves (Colwell and Coddington 1994; Moreno and Halffter 2000). The solution to this problem is a form of interpolation known as rarefaction. Rarefaction curves are constructed by repeatedly randomizing the order in which samples are added to the species accumulation curve and taking the mean values of cumulative species richness until a smooth curve is obtained (Longino and Colwell 1997). The rarefaction curve demonstrates the number of species that one would expect to find, on average, after x number of samples (Gotelli and Colwell 2001).

The Choctawhatchee River watershed in Alabama and Florida historically contained at least 21 native mussel species, of which one is now presumed extinct and five are federally threatened or endangered (TE) (Williams et al. 2008; USFWS 2012). We sampled mussels at three sites in the Choctawhatchee River watershed eight times each over 4 mo ($N = 24$). Two sites were in close proximity on the same stream (Eightmile Creek) to compare results at two similar locations. Our objectives were to (1) determine the number of samples needed to detect 80%, 90%, 95%, and 99% of the estimated total species richness at each site, and (2) determine what percentage of the estimated total species richness was detected after one to eight samples. The same analysis was performed on a subset of the community using only TE species due to their specific and limited habitat preferences (see Niraula et al. 2015a, 2015b, 2016). We also assessed species richness estimates as a function of the number of individuals encountered to allow application and comparison of our conclusions to other streams.

METHODS

Study Area

The Choctawhatchee River watershed is located in the Southeastern Plains Level III ecoregion of southeast Alabama and northwest Florida (USEPA 2013). The watershed covers approximately 12,297 km^2 and drains into Choctawhatchee Bay in Florida (Heath et al. 2010). We sampled three wadeable sites in the Choctawhatchee River watershed. All sites had predominantly sandy substrates typical of Gulf Coastal Plain streams, low to moderate amounts of woody debris, and depths generally less than 0.75 m. One site was located on the West Fork Choctawhatchee River at Blue Springs State Park, Barbour County, Alabama (BS, 31°39′49.6″N, 85°30′18.8″W), beginning about 10 m upstream of the bridge and extending 100 m upstream. This site was a fourth-order stream with an average width of 11.8 m. The second and third sites were located on a third-order stream, Eightmile Creek, Walton County, Florida. The second site (8M1, 30°58′50.3″'N, 86°10′45.5″W) began at the County Road 181 bridge and extended 68 m upstream with an average width of 6.3 m. The third site (8M2, 30°58′46.7″N, 86°10′45.4″W) was located about 75 m upstream of 8M1 (~150 m upstream of the County Road 181 bridge) and extended 40 m upstream with an average width of 6.3 m. Both streams had densely vegetated riparian zones and canopy cover.

We chose these sites because they supported diverse and abundant mussel assemblages including three federally threatened mussel species. Two additional endangered species were also documented historically at the West Fork Choctawhatchee River site (Pilarcyzk et al. 2006; Reátegui-Zirena et al. 2013). A total of eight species were reported at Eightmile Creek and 12 species were reported at BS (Pilarczyk et al. 2006).

Field Methods

We sampled each site using 5 person-hr timed searches for the initial sample. The area sampled on the initial visit was marked and mussels were sampled within the same reach at each subsequent visit, with each subsequent sampling occasion being approximately 5 person-hr. Sampling was conducted by searching all available habitat within the reach using a combination of visual searching and tactile probing at least 5 cm deep into the substrate (Carlson et al. 2008). Each site was sampled on two consecutive days at 1-mo intervals from June to October 2012 (for a total of eight sampling occasions), following Pollock's robust capture–recapture design (Pollock 1982). The Pollock design was used for a concurrent mark–recapture study at the same sites (Hyde et al. 2016), but the structure of the sampling design was not incorporated into this analysis. Mussels were identified and returned to the vicinity from which they were collected.

Data Analysis

We used the Chao 2 estimator to compute S_{est}, the estimated total species richness for each site (Chao 1987); this is a nonparametric estimator that makes no assumptions about the underlying population distribution and is commonly used to estimate species richness (Wei et al. 2010). We used the classic form of the Chao 2 estimator:

$$S_{est} = S_{obs} + \frac{q_1^2}{2q_2},$$

Table 1. Number of individuals needed to detect various percentages of estimated total mussel species richness at three sites in the Choctawhatchee River watershed, Alabama/Florida. *N* is the mean number of mussels/sample.

		% Estimated Total Species Richness			
Site	*N*	80	90	95	99
BS	121	310	550	732	921
8M1	509	—	1124	2780	4104
8M2	273	66	362	845	1665

where S_{est} is estimated total species richness, S_{obs} is detected species richness, and q_1 and q_2 are the number of uniques and duplicates, respectively. Uniques are species that were found in only one sample, and duplicates are species that were found in exactly two samples. We used this estimate to extrapolate a rarefaction curve past the reference sample (actual sampling effort, $N = 8$) using the formulas in the next paragraph. Thus, the curve to the left of the reference sample is the rarefaction curve (interpolation), while the curve to the right is the extrapolated curve.

The computer program EstimateS 9.0 (Colwell 2013) was used to calculate sample-based rarefaction curves using the following equation (equation 17 of Colwell et al. 2012):

$$\tilde{S}_{sample}(t) = S_{obs} - \sum_{Y_i>0}\left[\left(\frac{T - Y_i}{t}\right)/\left(\frac{T}{t}\right)\right],$$

where $\tilde{S}_{sample}(t)$ is the mean number of species expected in t subsamples from all T collected samples. The number of times each species was detected (i.e., incidence frequencies) is represented by Y_i, and S_{obs} is the total detected species richness. Curves were calculated for all three sites using number of samples ($N = 8$) and number of individuals as a measure of sampling effort. The following equation was used to extrapolate each rarefaction curve to 32 samples (equation 18 of Colwell et al. 2012):

$$\tilde{S}_{sample}(T + t^*) = S_{obs} + \hat{Q}_0\left[1 - \left(1 - \frac{Q_1}{Q_1 + T\hat{Q}_0}\right)^{t^*}\right],$$

where $\tilde{S}_{sample}(T + t^*)$ represents the number of species expected after $T + t^*$ samples, T is the total number of samples actually collected, and t^* is the number of additional samples to which one wishes to extrapolate. The number of species found in only one sample is represented by Q_1. The estimated number of species not found in any of the samples is represented by $\hat{Q}_0$. The Chao 2 estimator was used to estimate $\hat{Q}_0$ (equal to the second term from the Chao 2 estimator formula above), and the value computed from the above formula was used as the asymptote that each extrapolated curve approached.

Table 2. Number of 5 person-hr samples needed to detect various percentages of estimated total mussel species richness at three sites in the Choctawhatchee River watershed, Alabama/Florida. Percentages were calculated from the line of best fit in Figure 1. S_{obs} is the cumulative number of species detected; S_{est} is the estimated total species richness.

	% Estimated Total Species Richness					
Site	80	90	95	99	S_{obs}	S_{est}
BS	2.6	4.5	6.0	7.6	11	11.2
8M1	—	2.2	5.4	8.0	8	8.1
8M2	0.2	1.3	3.1	6.4	8	8.1

Table 3. Observed percentage of estimated total mussel species richness (Chao 2) detected after successive samples at three sites in the Choctawhatchee River watershed, Alabama/Florida. Percentages ≥90 are bolded. Small discrepancies between this table and Table 2 are a result of differences between observed percentages and predictions from fitted equations.

	Sample							
Site	1	2	3	4	5	6	7	8
BS	62	76	84	89	**93**	**95**	**97**	**98**
8M1	88	**90**	**91**	**93**	**94**	**96**	**97**	**99**
8M2	87	**93**	**96**	**98**	**99**	**99**	**99**	**99**

Rarefaction curves were used to determine the percentage of S_{est} sampled during each visit by dividing the cumulative number of expected species in t subsamples by the estimated total species richness of each site (S_{est}). We also fit a line to our rarefaction curves in Excel and used the resulting equation to calculate the expected number of samples needed to detect 80%, 90%, 95%, and 99% of S_{est}. The same analysis was done using only TE species to determine the sampling effort needed to detect 80%, 90%, 95%, and 99% of these species at BS. This calculation was not done for 8M1 and 8M2 because all three TE species were encountered on all eight sampling occasions at those sites.

RESULTS

A total of 7,222 mussels representing 11 species were collected over eight samples at all three sites. The cumulative number of mussel species detected after eight samples was eight at both 8M1 and 8M2, which is supported by historical findings of the same eight species at that location (Pilarczyk et al. 2006). The mean number of individuals in each sample was 509 at 8M1 and 273 at 8M2 (Table 1). The cumulative number of mussel species detected after eight samples was 11 at BS, where historical records show the same 11 species in addition to one federally endangered species, *Ptychobranchus jonesi* (Southern Kidneyshell), which we did not detect (Pilarczyk et al. 2006). The mean number of individuals in each sample at BS was 121.

Rarefaction curves indicated that 310 and 550 individuals were needed to detect 80% and 90%, respectively, of the estimated total species richness at BS (Table 1); given our sampling method and mussel abundance at this site, this translated to 2.6 and 4.5 samples, respectively (Table 2). Detection of 95% of estimated total species richness at BS

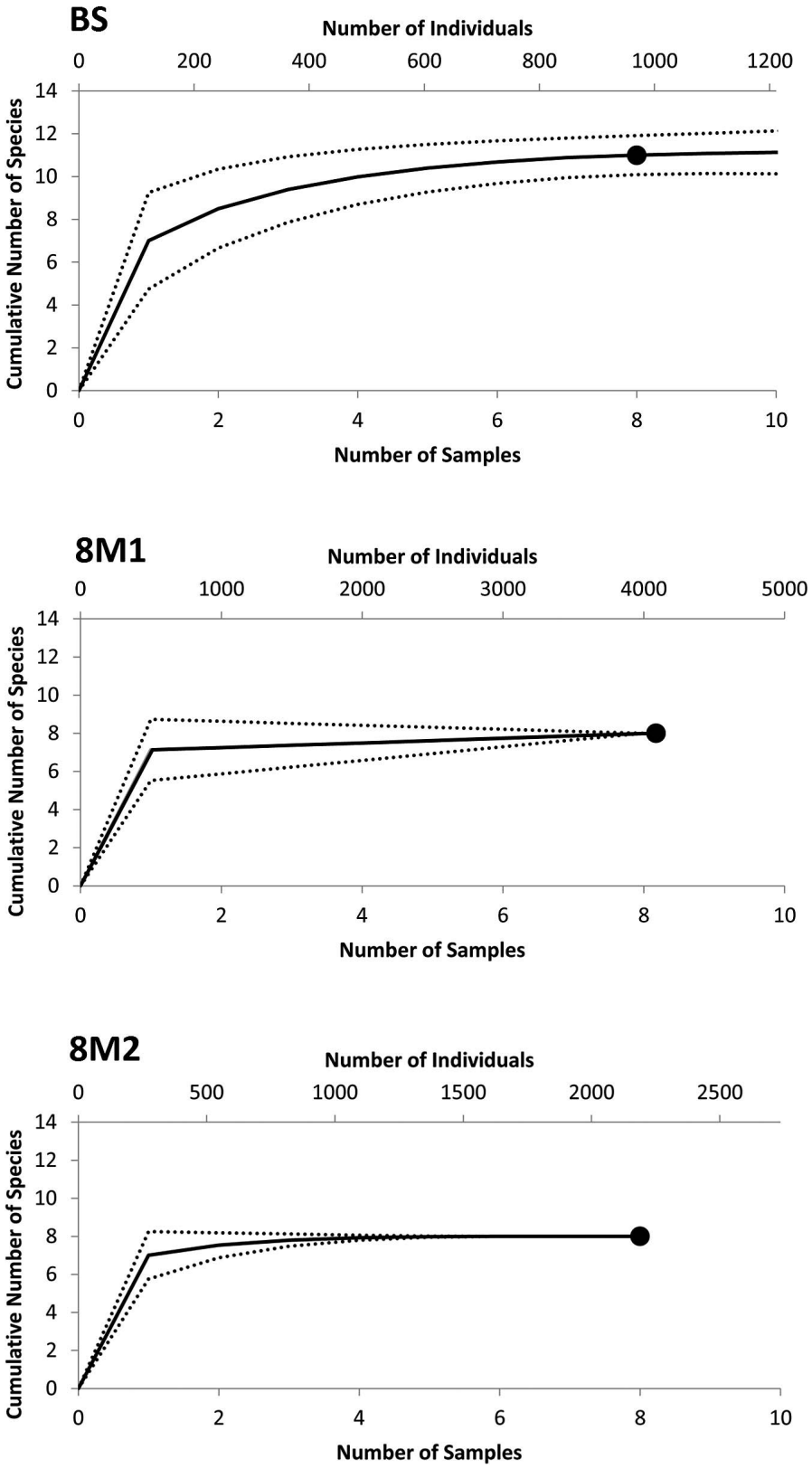

Figure 1. Rarefaction curves showing the cumulative number of species detected as a function of the number of samples and individuals collected at three sites in the Choctawhatchee River watershed, Alabama/Florida. Dotted lines are 95% confidence intervals.

required 732 individuals (6.0 samples). A single sample was sufficient to detect 80% of the estimated total species richness at both 8M1 (<510 individuals) and 8M2 (<273 individuals). Detection of 95% of the estimated total species richness at Eightmile Creek required a larger number of individuals but a smaller number of samples than at BS (8M1: 2,780 individuals, 5.4 samples; 8M2: 845 individuals, 3.1 samples).

Site BS had a much more gradual species accumulation curve than sites 8M1 and 8M2 (Table 3 and Fig. 1). With one sample, the percentage of estimated total species richness detected was much lower at BS (62%) than at 8M1 and 8M2 (88% and 87%, respectively), but percentage of detection converged for all three sites at around five samples. Percentage of detection reached only 98% at BS after all eight samples were taken. Both 8M sites had similar, steep species accumulation curves, but site 8M2 reached an asymptote after five samples, while 99% detection was not reached at 8M1 until eight samples were taken (Table 3 and Fig. 1).

All three TE species at 8M1 and 8M2 were found on all eight sampling occasions, indicating that one sample was sufficient to detect all the TE species at these sites. In contrast, only one TE species was found on all sampling occasions at BS (*Pleurobema strodeanum*, Fuzzy Pigtoe), and five samples were needed to detect *Fusconaia burkei* (Tapered Pigtoe). Only 45% of the estimated number of TE species (about two out of five of the historically recorded species) were detected at BS after one sample and only 90% (about four out of five species) were detected after all eight samples. An estimated 15.1 samples were needed to detect 99% of the federally listed species present at BS.

DISCUSSION

Local abundance is one of the primary influences on the number of samples needed to adequately estimate species richness. Blue Springs had lower abundance and higher diversity of mussels than the Eightmile Creek sites, with a correspondingly lower number of mussels per sample. A rarefaction study of fish in the Little Choctawhatchee River watershed found that very low abundance usually resulted in a lower percentage of species being detected for a given number of samples when compared with sites with higher abundance (Hyde et al. 2014). That study, along with our observations, suggests that higher abundance should result in higher species detectability. At least nine samples (electrofishing 35 times stream width) were needed to detect 90% of the estimated fish species richness based on a study of 12 sites, suggesting that mussel assemblages may require fewer repeat sampling events than fish to detect a similar percentage of species (Hyde et al. 2014). This difference likely is a result of fishes having greater mobility, decreased capture probability, and generally higher species richness.

A study in wadeable Illinois streams found means of 60.5%, 79.0%, and 87.4% of the estimated mussel species across 18 sites after 4, 10, and 14 person-hr, respectively (27–942 individuals and 5–18 species per site; Huang et al. 2011). These results are similar to our species richness estimates at BS after one, two, and three samples (62%, 76%, and 84%, respectively, 5 person-hr each), despite the fact that the Illinois study encompassed greater environmental variability and used an estimator based on abundance (Chao 1) rather than incidence data. Huang et al. (2011) also found that sampling adequacy decreased as stream size increased. This phenomenon may partially explain the lower number of samples needed to estimate species richness in Eightmile Creek, while the higher number of individuals needed at Eightmile Creek is likely a result of higher mean abundance per sample and the consequent lack of small sample sizes at those sites.

One sample (5 person-hr) was sufficient to find all TE species at both 8M1 and 8M2 because these species are locally abundant at the sites (Pilarcyzk et al. 2006; Reátegui-Zirena et al. 2013). At BS, TE species were much less common and greater effort was necessary to detect them. The Chao 2 estimator predicted 4.4 TE species at BS and five TE species were reported historically from this site, but we found only four TE species. The only TE species we did not find was the federally endangered *Ptychobranchus jonesi*; this species is on the verge of extinction and only a few individuals have been found in the last 20 yr with the exception of Gangloff and Hartfield (2009) who found 13 individuals in Pea River (Blalock-Herod et al. 2005; Pilarcyzk et al. 2006; Williams et al. 2008). In another study, increasing sampling effort from 1.5 to 4.5 person-hr at a site dramatically increased detection of rare mussel species, but even this increased effort was not sufficient to consistently detect extremely rare species (Metcalfe-Smith et al. 2000). Our model predicted that 15 samples were necessary to detect all species at BS, but for extremely rare species such as *P. jonesi*, detection is largely a matter of chance.

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