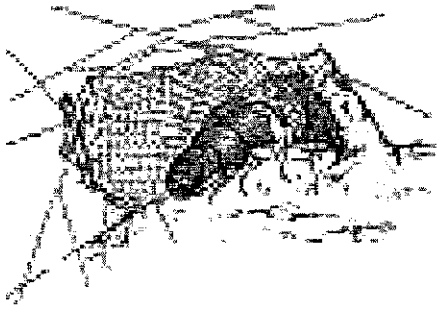
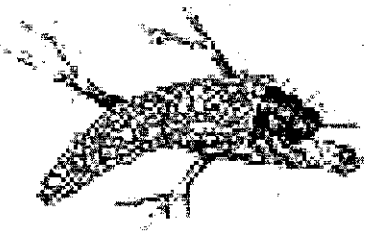
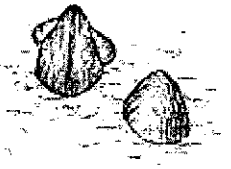
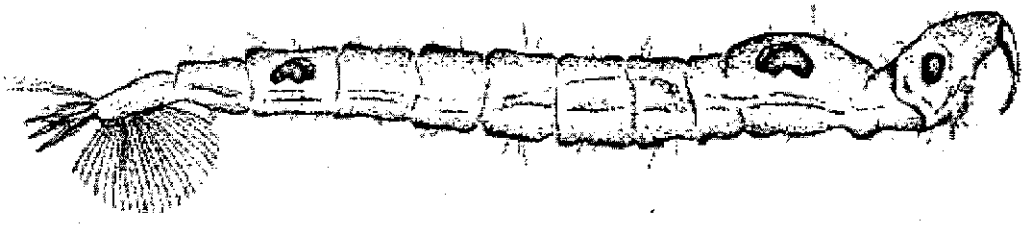
COMPARISON OF 100-ORGANISM VS. 300-ORGANISM SUBSAMPLING FOR USE WITH ADEQ'S AQUATIC MACROINVERTEBRATE SAMPLING METHODOLOGY









ARKANSAS DEPARTMENT OF ENVIRONMENTAL QUALITY

WATER DIVISION

REPORT WQ03-02-1

COMPARISON OF 100-ORGANISM VS. 300-ORGANISM SUBSAMPLING FOR **USE WITH ADEQ'S AQUATIC MACROINVERTEBRATE SAMPLING METHODOLOGY**

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Water Division
Little Rock, Arkansas

Report WQ03-02-1

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INTRODUCTION

The Arkansas Department of Environmental Quality (ADEQ) has utilized bioassessments since the 1970's as a technique to evaluate and monitor water quality and ecological health of aquatic systems. Shackleford (1988) was a pioneer in biocriteria development utilized by ADEQ in monitoring water quality in Arkansas. More recently, Davidson and Clem (in review) have focused attention on refining biocriteria in Arkansas based on regional specificity. Davidson and Clem (in review) analyzed the classification of streams using multivariate ordination, box-and-whisker plots and correlation analyses to select biological metrics that were ecologically relevant to Arkansas streams. The most responsive metrics to independent (but imprecise) measures of disturbance were aggregated into an index of biological integrity (IBI) for small watersheds (< 80 km^2). Data is currently being collected in larger wadeable streams to develop an IBI, evaluate if a difference exists between smaller vs. larger wadeable streams, and evaluate the effects of seasonality on the IBI.

Considerable documentation exists validating the effectiveness of bioassessments in lotic streams to monitor environmental quality in streams throughout the world. Nevertheless, subsampling is still a source of much controversy among managers and biologists. Much of this debate has arisen from the use of fixed-count subsampling, a method in which random cells in a sorting pan are picked until a target number of organisms (e.g. 100) is obtained. ADEQ biologists utilize a modification of this method. Fixed-count subsampling, particularly 100-organism count recommended by the original EPA Rapid Bioassessment Protocols of Plafkin et al. (1989), has been criticized. Justus et al. (2001) suggest that some intersite discriminating ability for some metrics may be lost due to subsample size (100-organism) utilized by ADEQ. Courtemanch (1996) claimed that 100-organism subsamples provide unstable estimates of taxon richness and destroy any consistency of areal sample size. However, others have maintained the cost-saving benefits of fixed-count processing outweigh the potential loss of information, with some studies showing that even 100-organism bioassessments were able to distinguish sites of differing ecological impairment and produce stable values for metrics when compared to larger counts (Barbour and Gerritsen, 1996).

The objective of this study was to address the issue, effectiveness and implications of increasing ADEQ subsample size from 100-organism to a more widely accepted 300-organism sample size. The questions addressed included 1) is there a significant difference between metric values for subsample size, 2) how much additional time/costs are associated with increasing sample size, 3) does the benefit outweigh the costs, and 4) is our ability to properly assign a rating category in the IBI hindered by smaller sample sizes.

METHODS

Two riffles in each of six Ouachita Mountain streams, for a total of 12 samples, were sampled between April 23 and May 2, 2002. The Ouachita Mountains are located in the west-central portion of the state. The Ouachitas are composed of severely folded and faulted sandstones, shales and novaculite. In addition, there are some outcroppings of igneous rock and interbedded limestone. Topography ranges from rolling hills to very steep, rugged terrain with some slopes exceeding 50 percent. Forests are composed of mixed hardwood and pine. Silviculture, pasture and poultry production are major land uses. Watershed sizes for the streams surveyed ranged from 24 to 125 mi^2. Five of six sites were considered to be least-disturbed reference sites, while the sixth was impaired by nutrients from sewage effluent and golf course runoff.

Five minute aquatic macroinvertebrate samples were collected using a traveling kick method and 30.5 cm D-shaped dip net. The five-minute kick was conducted throughout the riffle. After collecting, the sample was washed through a sieve; all large organic and inorganic debris was removed, and placed in a 1.0 L jar. Samples were preserved in 70% ethanol to be transported to the lab for identification and enumeration.

In the lab, the sample was placed into an 8 x 13 inch dissecting pan. The pan was swirled to evenly distribute the sample and a 4-inch (10 cm) diameter ring randomly placed on the sample. Organisms were removed from the ring until the ring was depleted of organisms. If less than 95 organisms were encountered during the 100-organism subsample, the sample was swirled again and the ring randomly replaced. The same procedure was followed until a minimum of 95 organisms was removed from the sample. Once the 100-organism subsample was completed, the same procedure continued until a 300-organism subsample (100 organisms plus an additional 200 organisms) was obtained. One hundred-organism samples were placed in a separate jar from 300-organism samples. Processing times were recorded separately for each subsample.

One person conducted taxonomic determinations to assure consistency. Organisms were identified to the lowest feasible taxonomical level, typically genus level, using keys by Merritt and Cummins (1996) and Pennak (1978). Taxa determinations were checked against regional data to ensure accuracy. Taxa, raw tallies and identification times were recorded on bench sheets for each subsample and entered into an ADEQ database for further analysis.

RESULTS

Table 1 shows range and mean processing times for 100-organism and 300-organism subsamples. Processing times for individual samples are presented in Appendix 1. Total processing time is separated into three components, sample collection, subsampling and identification, to show differences and the impact of each component on total processing time. Since both the 100-organism and 300-organism subsamples were taken from the same collection, collection time was the same for both subsamples and has no influence on differences observed regarding total processing time. The average time required to pick a 300-organism subsample was 2.3 times greater than that required for a 100-organism subsample. Identification times increased an average of 0.55 man-hour (1.8X) for 300-organism subsamples. Picking and

identification time was increased approximately 1 man-hour in order to obtain a 300-organism subsample when compared to a 100-organism subsample.

The same biologist collected, subsampled and identified all samples. Therefore, sampling times were not influenced by the processor, but rather by the number of individuals and uncommon taxa that required more time for proper identification.

Table 1. Range and mean processing effort and number of individuals sampled for 100-organism and 300-organism subsamples from 12 samples in the Ouachita Mountains, 2002.

	100-Organism Subsample		300-Organism Subsample	
Description	**Range**	**Mean**	**Range**	**Mean**
Processing time				
Collection	0.20 - 0.38	0.30	0.20 - 0.38[a]	0.30[a]
Subsample	0.19 - 0.36	0.27	0.50 - 0.76	0.63
Identification	0.40 - 1.02	0.71	0.75 - 2.09	1.26
Total[b]	0.96 - 1.71	1.28	1.67 - 3.08	2.19
Number of individuals sampled	93 - 221	152	255 - 364	310

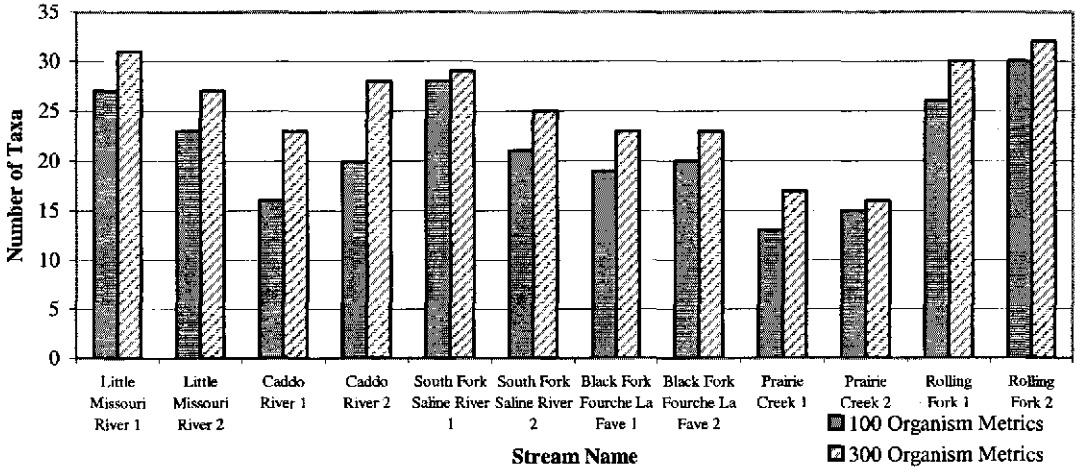
[a] 100-organism and 300-organism samples were obtained from the same samples. Therefore, collection time will be the same for each subsample.
[b] Total processing time is the sum of collection, subsample and identification times.

One hundred-organism metric values for 14 metrics were similar to 300-organism metric values (Appendix 2). Number of taxa, EPT and intolerant taxa increased with increased subsample size. Composition, tolerance and trophic measures were variable, either increasing or decreasing with subsample size. Figures 1a to 1j illustrate metric comparisons for 100-organism versus 300-organism subsampling.

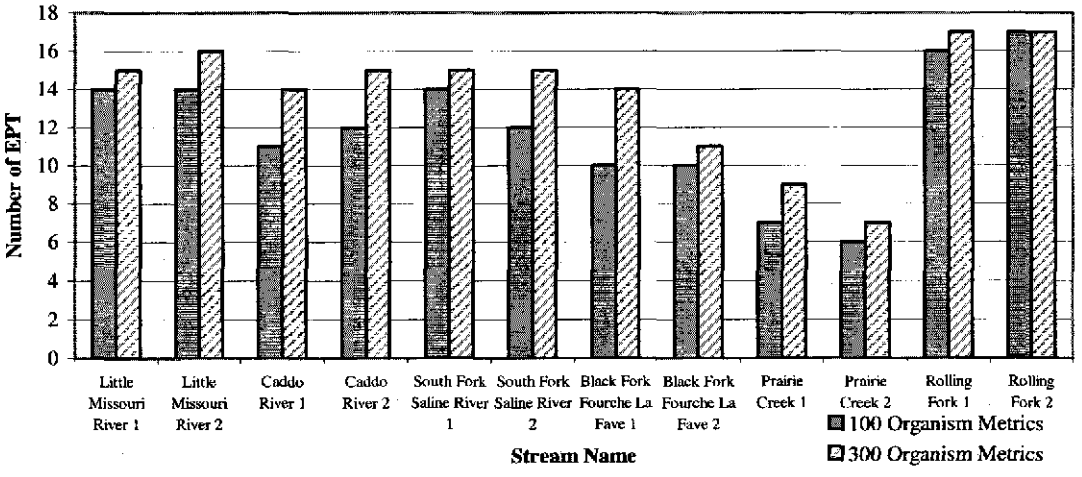
Analysis of variance (ANOVA) was tested on seven metrics and an IBI commonly used by ADEQ. Results from the ANOVA are presented in Table 2 and indicate that no significant difference occurred between 100-organism and 300-organism metrics. Richness measures had the greatest observed variability. Although no significant difference was inferred, taxa richness was influenced the greatest by subsample size. One taxon represented 63 percent of taxa added by increasing subsample size. Additional taxa reported in 300-organism subsamples represented less than 9% of the total community. On average, additional taxa in 300-organism subsamples represented 2.6% of the total community.

Subsample size had little, if any, impact on discriminatory ability using an ADEQ IBI. One site, 8.3% of samples, received a better water quality rating as a result of using 300-organism subsample size compared to 100-organism subsample size. IBI scores and ratings are presented in Table 3.

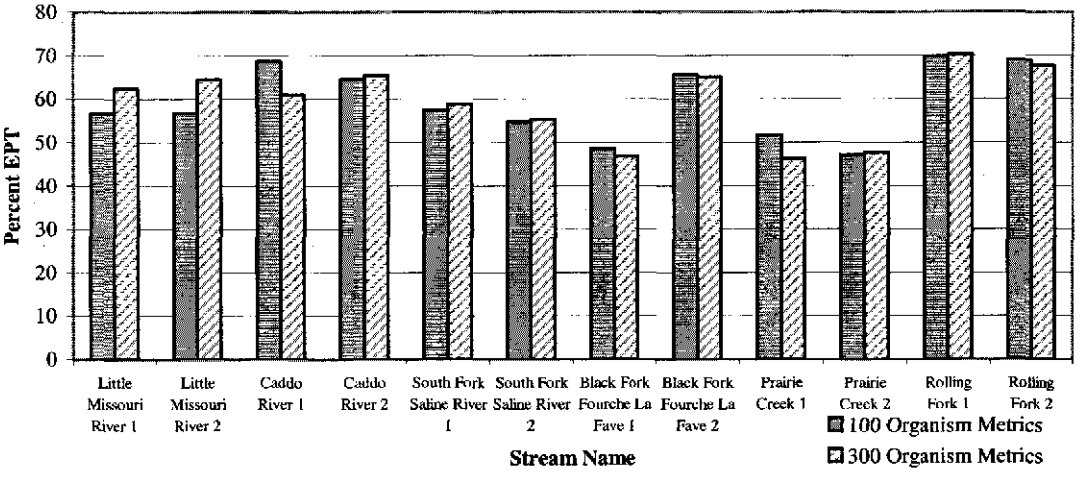
1a. Taxa Richness



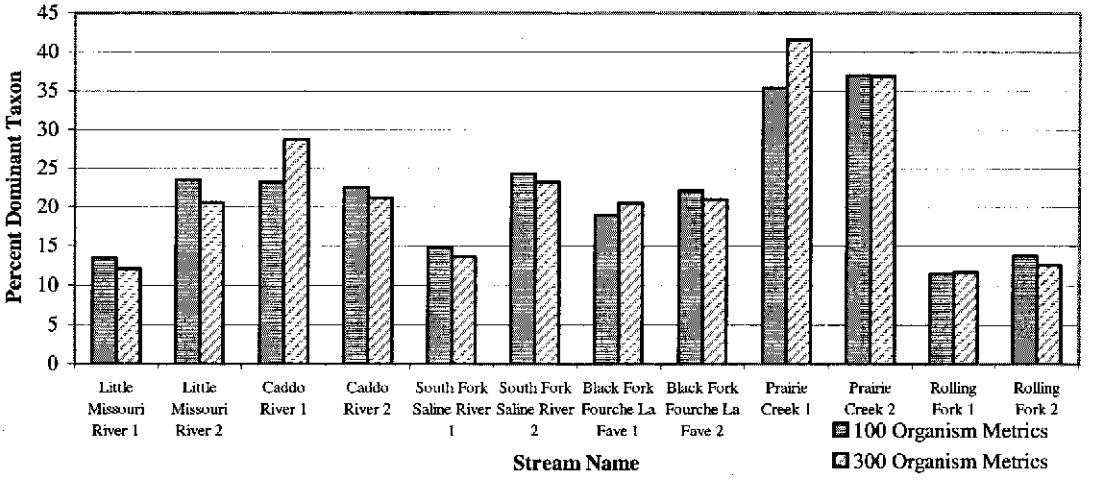
1b. EPT Taxa



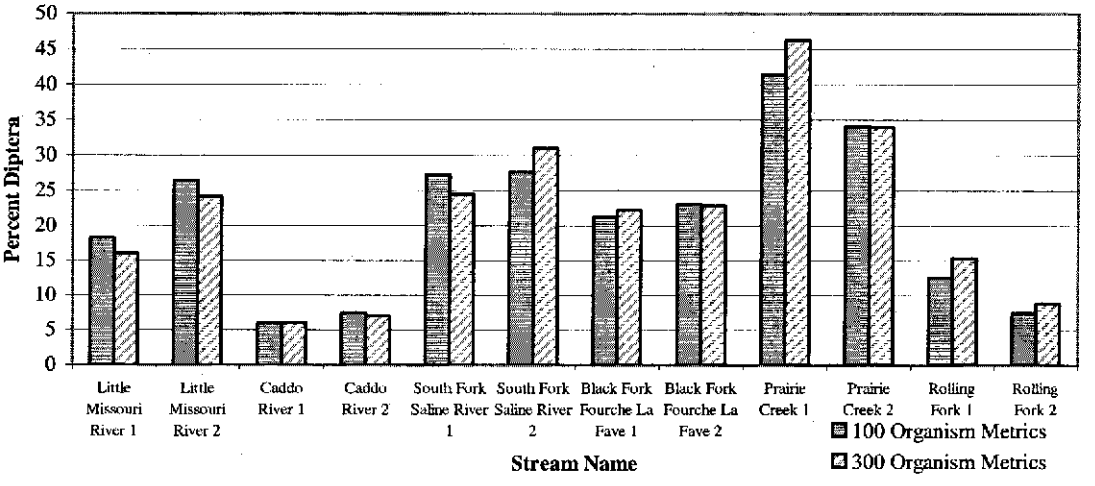
1c. EPT Composition



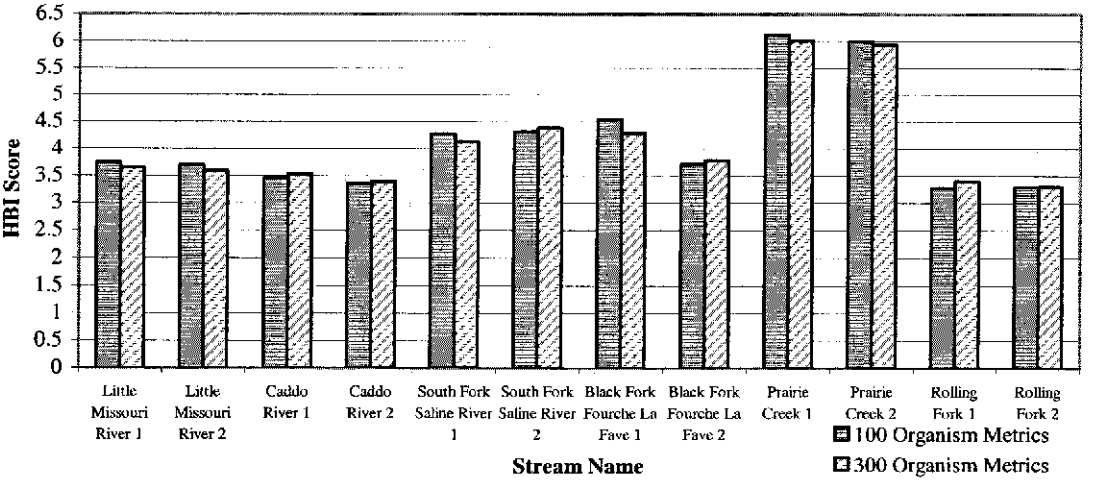
1d. Dominant Taxon Composition



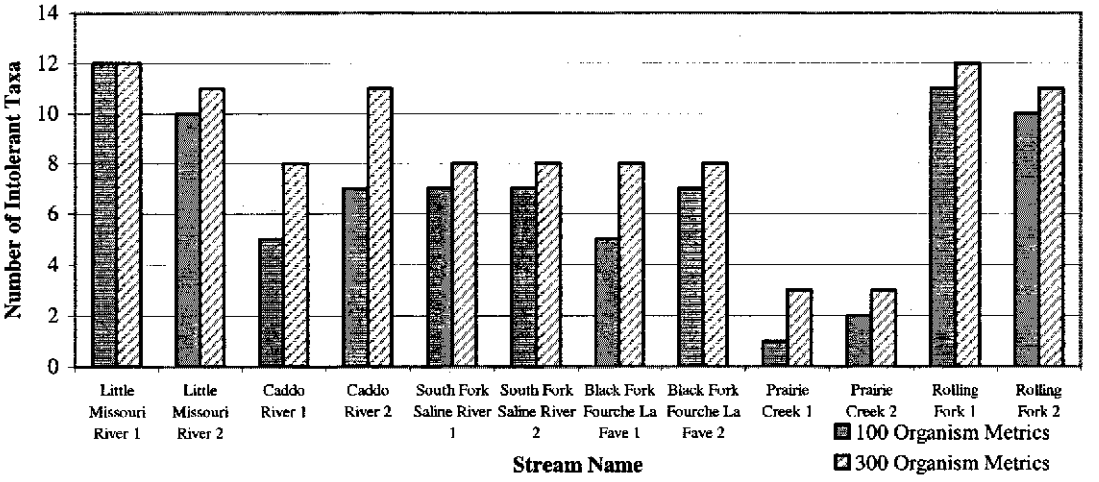
1e. Diptera Composition



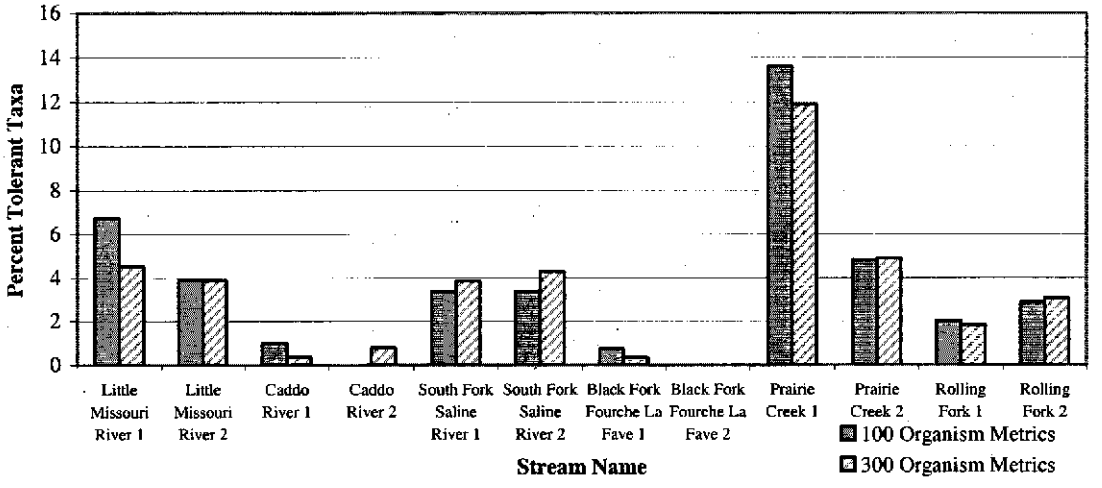
1f. Hilsenhoff Biotic Index



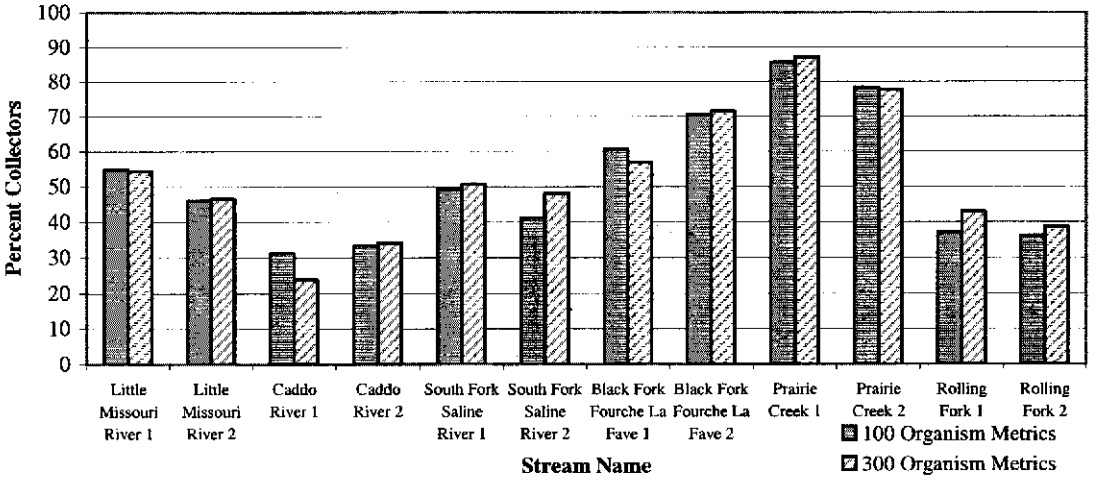
1g. Intolerant Taxa



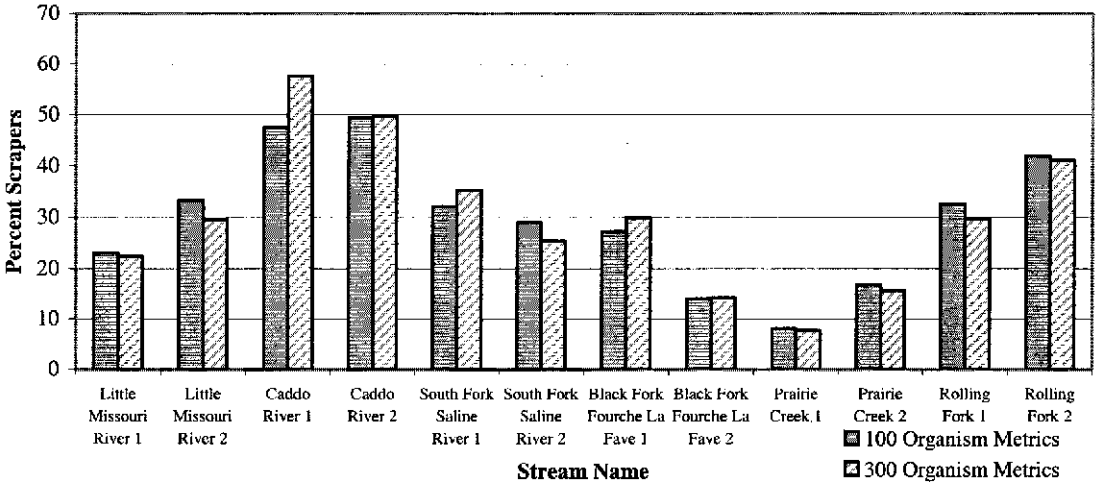
1h. Tolerant Taxa Composition



1i. Collector Composition



1j. Scraper Composition



1k. Filterer Composition

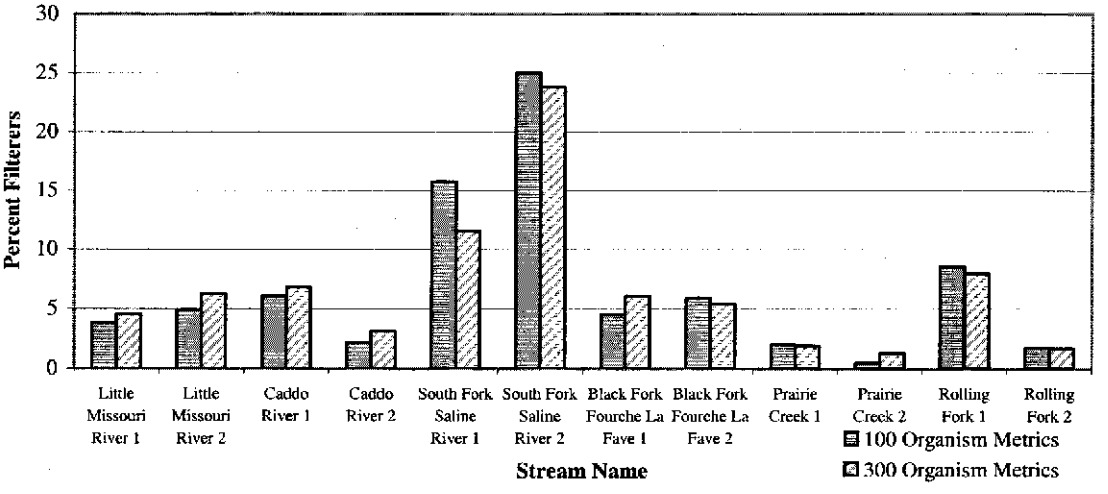


Table 2. Analysis of variance results from 100-organism vs. 300-organism subsamples for seven commonly used metrics and one IBI used by ADEQ.

Metric	SS	df	MS	F	P-value	F crit
Taxa Richness	88.16	1	88.17	3.14	0.09	4.30
EPT Richness	20.16	1	20.17	1.92	0.18	4.30
No. Diptera	1.04	1	1.04	0.74	0.40	4.30
% Diptera	1.06	1	1.07	0.01	0.93	4.30
% Dominant Taxa	0.37	1	0.37	0.00	0.95	4.30
% Collectors	2.71	1	2.71	0.01	0.93	4.30
Hilsenhoff Biotic Index	0.00	1	0.01	0.01	0.94	4.30
IBI*	0.17	1	0.17	0.00	0.96	4.30

Table 3. IBI scores and ratings for 100-organism and 300-organism subsamples.

100-Organism IBI Score	300-Organism IBI Score	100-Organism Rating	300-Organism Rating
28	28	Very Good	Very Good
30	30	Very Good	Very Good
32	32	Very Good	Very Good
32	34	Very Good	Very Good
28	28	Very Good	Very Good
30	26	Very Good	Good/Very Good
22	26	Good	Good/Very Good
24	28	Good	Very Good
10	10	Poor	Poor
8	8	Very Poor/Poor	Very Poor/Poor
34	30	Very Good	Very Good
34	34	Very Good	Very Good

SUMMARY

Biological metrics and biocriteria need to be effective discriminators of water quality impairments. Subsampling size is a controversial issue and probably will continue to be a topic of controversy surrounding rapid bioassessments. For this reason, it is important to substantiate ADEQ's bioassessment protocols with reliable scientific evidence that validates the Department's current methodology for subsampling aquatic macroinvertebrate samples.

Results presented in this report did not support increasing subsample size from 100-organisms to 300-organisms. Some factors observed that validate ADEQ's use of 100-organism subsampling include:

- No significant difference was observed between metric values for 100-organism versus 300-organism subsamples;
- Richness measures increase, although not significantly, with increased subsample size;
- The effects of subsample size on composition and tolerance measures are variable;
- Subsample size has little, if any, effect of discriminatory ability. Only 8% of sites received a higher IBI rating that resulted in a rating increase from good to very good;
- Subsample size and time required to properly identify uncommon taxa influence total processing time when a single processor is used;

Some factors not examined during this study that may influence processing time include the use of multiple processors, seasonality, collection method and ecoregion. Multiple processors may not have the same experience and/or expertise that may result in more variability in processing samples. Typically, more time is required to pick fall samples because of a greater amount of organic material from new leaf fall. ADEQ utilizes a different collection method in lowland streams of the Gulf Coastal Plain and Delta. Based on best professional judgment, we would

expect there to be a greater difference for subsample time when processing samples from these two ecoregions and from the fall collection period. This would be a result of increased difficulty associated with locating and picking organisms from samples with a greater amount of organic matter.

An additional man-hour was required for 300-organism subsamples. Currently, ADEQ is collecting 100 to 150 aquatic macroinvertebrate samples per year. Increasing subsample size to 300-organisms would require 100 to 150 additional man-hours per year at a cost of $2,000 to $3,000 for labor (based on $20/man-hour). This additional requirement for labor is not justified at this time based on results presented within this report.

It is our recommendation that since current data indicates no obvious advantage to increasing subsample size and labor that the Department retain its current subsample size of 100-organisms.

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APPENDIX 1

PROCESSING TIMES, RINGS PICKED, NUMBER OF INDIVIDUALS AND NUMBER OF TAXA FOR SAMPLES PROCESSED FROM OUACHITA MOUNTAIN STREAMS

Appendix 1. Processing times, rings picked, number of individuals and number of taxa for samples processed from Ouachita Mountain streams, 2002.

Stream Name	Sample #	Reach Length (ft)	Reach Width (ft)	Area (ft^2)	Collection Time
S.F. Saline River	1	123	100	12300	0.27
S.F. Saline River	2	90	40	3600	0.23
Prairie Creek	1	25	55	1375	0.35
Prairie Creek	2	43	60	2580	0.36
Little Missouri River	1	86	126	10836	0.31
Little Missouri River	2	51	131	6681	0.26
Black Fork Fourche La Fave	1	111	68	7548	0.33
Black Fork Fourche La Fave	2	109	78	8502	0.24
Rolling Fork	1	59	40	2360	0.38
Rolling Fork	2	130	29	3770	0.32
Caddo River	1	135	32	4320	0.30
Caddo River	2	141	45	6345	0.20
Averages		92	67	5851	0.30

Stream Name	Picking Time (100)	Add. Time To 300	Picking Time (300)	Number of Rings (100)	Add. Number of Rings (300)
S.F. Saline River	0.36	0.18	0	1	1
S.F. Saline River	0.23	0.39	0	1	2
Prairie Creek	0.28	0.29	0	1	1
Prairie Creek	0.29	0.29	0	1	1
Little Missouri River	0.25	0.43	0	1	2
Little Missouri River	0.19	0.45	0	1	3
Black Fork Fourche La Fave	0.32	0.37	0	2	3
Black Fork Fourche La Fave	0.19	0.45	0	1	4
Rolling Fork	0.31	0.30	0	1	1
Rolling Fork	0.29	0.21	0.00	2	2
Caddo River	0.28	0.48	0	2	3
Caddo River	0.28	0.40	0	3	entire sample
Averages	0.27	0.35	0.00	1.42	2.09

*Units of time are reported as man-hour(s).

Appendix 1. Processing times, rings picked, number of individuals and number of taxa for samples processed from Ouachita Mountain streams, 2002.

Stream Name	ID Time (100)	Add. ID Time (300)	ID Time (300)	Total Processing Time (100)	Total Processing Time (300)
S.F. Saline River	1.20	0.31	1.51	1.83	2.32
S.F. Saline River	0.99	0.63	1.09	0.92	1.94
Prairie Creek	0.40	0.35	0.75	1.03	1.67
Prairie Creek	0.54	0.23	0.77	1.19	1.71
Little Missouri River	0.97	1.12	2.09	1.53	3.08
Little Missouri River	0.51	0.95	1.46	0.96	2.36
Black Fork Fourche La Fave	0.70	0.59	1.29	1.35	2.31
Black Fork Fourche La Fave	0.91	0.31	1.22	1.34	2.10
Rolling Fork	1.02	0.49	1.51	1.71	2.50
Rolling Fork	0.84	0.43	1.27	1.45	2.09
Caddo River	0.45	0.59	1.04	1.03	2.10
Caddo River	0.52	0.65	1.17	1.00	2.05
Averages	0.71	0.55	1.26	1.28	2.19

Stream Name	# Individuals Sampled (100)	# Individuals Sampled (300)	# Taxa (100)	# Taxa (300)
S.F. Saline River	210	295	28	29
S.F. Saline River	138	364	21	25
Prairie Creek	147	320	13	17
Prairie Creek	208	310	15	19
Little Missouri River	104	330	27	31
Little Missouri River	102	385	23	27
Black Fork Fourche La Fave	132	297	19	23
Black Fork Fourche La Fave	221	315	20	23
Rolling Fork	199	326	26	30
Rolling Fork	174	294	30	32
Caddo River	99	273	16	23
Caddo River	93	255	20	28
Averages	152	310	22	25

*Units of time are reported as man-hour(s).

APPENDIX 2

SELECTED METRIC RESULTS FOR 100-ORGANISM AND 300-ORGANISM SUBSAMPLES COLLECTED FROM OUACHITA MOUNTAIN STREAMS

Appendix 2. Selected metric results for 100-organism and 300-organism subsamples collected from Ouachita Mountain streams, 2002.

Station ID	Richness Measures		Composition Measures				Tolerance Measures			Trophic Measures				
	No. Taxa	No. EPT	% Dom. Taxon	Percent EPT	Percent Diptera	Percent Chiron.	HBI	No. Intol.	% Tol. Taxa	Percent Shredder	Percent Collector	Percent Filterer	Percent Scraper	Percent Predator
100 Organism Metrics														
Little Missouri River 1	27	14	13.46	56.73	18.27	13.46	3.25	12	6.75	4.81	54.81	3.85	23.08	13.46
Little Missouri River 2	23	14	23.53	56.86	26.47	24.51	3.7	10	3.92	7.84	46.08	4.9	33.33	7.84
Caddo River 1	16	11	23.23	68.69	6.06	4.04	3.45	5	1.01	2.02	31.31	6.06	47.47	13.13
Caddo River 2	20	12	22.58	64.52	7.53	6.45	3.35	7	0.00	2.15	33.33	2.15	49.46	12.9
South Fork Saline River 1	28	14	14.83	57.42	27.27	12.92	4.27	7	3.35	0.00	49.28	15.79	32.06	2.87
South Fork Saline River 2	21	12	24.32	54.73	27.70	3.38	4.31	7	3.38	1.35	41.22	25	29.05	3.38
Black Fork Fourche La Fave 1	19	10	18.94	48.49	21.21	19.70	4.54	5	0.76	0.00	60.61	4.55	27.27	6.82
Black Fork Fourche La Fave 2	20	10	22.17	65.61	23.08	21.72	3.72	7	0.00	0.00	70.59	5.88	14.03	9.05
Prairie Creek 1	13	7	35.37	51.7	41.50	37.41	6.12	1	13.61	2.72	85.71	2.04	8.16	1.36
Prairie Creek 2	15	6	37.02	47.12	34.13	32.69	5.99	2	4.81	0.00	78.37	0.48	16.83	4.33
Rolling Fork 1	26	16	11.56	69.85	12.56	8.04	3.28	11	2.01	11.06	37.19	8.54	32.66	10.55
Rolling Fork 2	30	17	13.79	68.97	7.47	4.60	3.29	10	2.87	4.60	36.21	1.72	41.95	14.94
300 Organism Metrics														
Little Missouri River 1	31	15	12.08	62.45	16.01	12.08	3.65	12	4.53	4.53	54.38	4.53	22.56	12.69
Little Missouri River 2	27	16	20.6	64.48	24.18	21.19	3.59	11	3.88	4.48	46.57	6.27	29.55	13.13
Caddo River 1	23	14	28.79	60.98	6.06	3.03	3.53	8	0.38	1.52	23.86	6.81	57.58	10.23
Caddo River 2	28	15	21.18	65.49	7.06	5.88	3.39	11	0.78	1.57	34.12	3.14	49.8	11.37
South Fork Saline River 1	29	15	13.64	58.74	24.48	13.99	4.13	8	3.85	0.35	50.7	11.54	35.31	2.1
South Fork Saline River 2	25	15	23.26	55.35	31.02	7.75	4.38	8	4.28	1.34	48.13	23.8	25.4	1.34
Black Fork Fourche La Fave 1	23	14	20.54	46.8	22.22	20.88	4.29	8	0.54	0.34	56.9	6.06	29.97	6.4
Black Fork Fourche La Fave 2	23	11	20.95	65.08	22.86	21.9	3.78	8	0	0	71.43	5.4	14.29	8.57
Prairie Creek 1	17	9	41.56	46.25	46.25	43.75	6.01	3	11.88	1.25	87.19	1.88	7.81	1.88
Prairie Creek 2	16	7	36.93	47.71	33.99	32.03	5.94	3	4.9	0	77.78	1.31	15.68	5.23
Rolling Fork 1	30	17	11.66	70.25	15.34	11.04	5.4	12	1.84	8.28	42.94	7.98	29.75	11.04
Rolling Fork 2	32	17	12.59	67.69	8.84	6.8	3.3	11	3.06	4.76	38.78	1.7	41.16	13.27