Layzer
Madison
1996

DRY TISSUE WEIGHT AS AN INDICATOR OF MUSSEL CONDITION — A CAUTIONARY NOTE

JAMES B. LAYZER and LESA M. MADISON
Tennessee Cooperative Fishery Research Unit
Tennessee Technological University
PO Box 5114
Cookeville, TN 38505

ABSTRACT

The spread of zebra mussels threatens the continued existence of many species of native unionids. Because it is unknown if breeding populations of native unionids can be maintained long-term (>10 yrs) in captivity, management agencies are faced with making a decision as to if and when unionids should be brought into captivity. We examined the use of dry tissue weight as an indicator of mussel condition. We collected 48 to 57 *Fusconaia ebena* each month over a 1 yr period from the same site in Kentucky Lake to determine seasonal variation in tissue weights. Samples of *F. ebena* were also collected from four other sites on the Tennessee River during late-August or early September in 1993 and 1994 to assess among site and between year differences. All regressions of dry tissue weight on dry shell weight or shell length were significant. Analysis of covariance indicated significant differences ($P<0.05$) among some sites and months. Because our samples of *F. ebena* were not infested with zebra mussels, these seasonal and site differences in tissue weight reflect normal variation and may be due to reproductive activity or other factors. We recommend that potential seasonal and site differences be investigated for other methods of determining mussel condition before management recommendations are made.

Introduction

Zebra mussels (*Dreissena polymorpha*), an exotic species from Europe, were introduced into the Laurentian Great Lakes in 1985 or 1986 (Griffiths et al. 1991; Hebert et al. 1989). This exotic species has devastated mussel populations in western Lake Erie (Schloesser and Nalepa 1994) and Lake St. Clair (Gillis and Mackie 1994). Zebra mussels attach to the exposed part of the unionid's shell (posterior end) and can prevent the unionid from fully opening or closing its valves. Possible effects of zebra mussel infestations on unionids include: impaired locomotion and burrowing, exposure to predators, and interference with respiration, feeding, growth, and reproduction (Mackie 1991).

Since introduction into the Great Lakes, zebra mussels have rapidly spread down the Mississippi and Illinois rivers. Impacts from zebra mussels could eventually cause major declines in all native unionid populations; but currently biologists are concerned with the continued existence of big river mussels, notably the imperiled and commercially important species. The discovery of a zebra mussel during September 1991 near Tennessee River Kilometer (TRKm) 48 raised concerns that the shell industry in Kentucky Lake, which annually contributes an estimated $40 million to the economy of Tennessee (Todd 1993), was threatened. Biologists also feared that some endangered species would be severely impacted by the probable rapid expansion of zebra mussels throughout the Tennessee River.

The last remaining populations of some endangered species, such as *Plethobasus cooperianus*, seem most threatened because they only occur in the Tennessee River and other large, navigable rivers where the impacts from zebra

mussels are expected to be greatest. To prevent the extinction of endangered species living only in big rivers, it may be necessary to establish captive populations and thus, a refugia from zebra mussels. Unfortunately, maintaining mussels in captivity has only recently been attempted, and conditions that promote high survival are poorly understood and may be species specific (see Dunn and Layzer, and Naimo et al., these proceedings). In light of the uncertain survival of endangered species held in captivity, biologists are faced with the dilemma of attempting to decide when to "rescue" endangered species from zebra mussels. Clearly, heavy infestations of zebra mussels result in mortality of unionids (Nalepa 1994); however, the level of infestation that would significantly reduce survival of unionids brought into captivity is less clear. Presumably, not only the infestation level but also the length of time of infestation would influence subsequent survival.

We were interested in determining how the condition of native mussels would be affected as the population of zebra mussels expanded in the Tennessee River. Fishery biologists have long used weight-length relationships to describe fish condition and to gain insight into factors affecting populations such as seasonal differences or those related to reproduction. With some exceptions (e.g. Payne and Miller 1989), relatively few studies of mussel populations have examined such relationships. Hence, we wanted to determine if weight-length relationships could be used to describe unionid condition in the Tennessee River before and after the spread of zebra mussels.

The objectives of our study were to establish baseline data on unionid densities, species composition, and weight-length relationships for *Fusconaia ebena*

in the lower Tennessee River prior to a seemingly imminent threat from zebra mussels. In this paper, we examine the variation in weight-length relationships among seasons, locations, and years.

Materials and Methods

We collected mussels by SCUBA diving at five sites on the lower Tennessee River to monitor the effects of zebra mussels on unionids: Site 1 was located in the tailwaters of Kentucky Dam at river kilometer (RKm) 29.3; Site 5 was located in the tailwaters of Pickwick Dam at RKm 316; Site 2 (RKm 108), Site 3 (Rkm 165), and Site 4 (RKm 175) were located in Kentucky Lake (Figure 1).

Site 3 was sampled monthly for one year (beginning in November 1993) to only determine possible seasonal variation in the relationship between tissue dry weight (TDW) and shell dry weight (SDW). Monthly samples collected at Site 3 contained from 48 to 57 *Fusconaia ebena* and an attempt was made to collect a representative size range of mussels. Adductor muscles were severed before preserving specimens in 10% buffered formalin. In the laboratory, mussels were soaked in water for 3 to 7 days to reduce the formalin odor and the outside of the shells were cleaned with a scrub pad to remove algae and sediment. All tissue was removed from the shell and placed in a preweighed, numbered aluminum pan. The pans were placed in a preheated 65°C oven, dried for 48 hours, weighed, and the tissue was discarded. Shells were permanently labeled with identification numbers on both valves and length and height of each shell were measured with dial calipers to the nearest mm. Shells were air-dried for 21 days and then weighed to the nearest 0.001 g.

The remaining four sites were sampled in late August or early September 1993 and 1994 to determine unionid densities, relative abundance of species, and between year and among site variation in the relationship of TDW to SDW. At each of these quantitative sampling sites, 20 quadrat (0.25 m^2) samples were collected by hand excavating the substrate to a depth of about 10 cm. All excavated material was placed into 6 mm mesh dive bags, brought to the surface, and sieved. Except for some small (<10 mm long) juvenile unionids, all individuals were identified and measured with dial calipers.

All measurements were transformed to common logarithms prior to analysis. Slopes from least squares regressions of tissue dry weight on shell dry weight or shell length were compared by analysis of covariance (Steele and Torrie 1980). If slopes did not differ significantly ($P>0.05$), a common slope was calculated and mean tissue weights adjusted for shell weight were compared.

Results

In 1993, unionid densities ranged from 11.8/m^2 at site 2 to 70.6/m^2 at site 5 (Table 1). Density and species richness were greatest at the two riverine sites (1 and 5). At all sites, *Fusconaia ebena* was one of the most abundant species collected. There were no zebra mussels attached to any of the unionids we collected. We also made a qualitative search at each site and we did not find any zebra mussels attached to 739 mussels examined. In 1994, we collected 4,816 mussels for various purposes at these same sites and found only one zebra mussel attached to a rock. Thus, even though the first zebra mussel was found in 1991, their population was negligible in Kentucky Lake throughout our study.

All least squares regressions of tissue dry weight on shell dry weight or shell length were highly significant (P=0.0001). Results of analysis of covariance indicated that the slopes for the regressions of $\log_{10}$ tissue dry weight on $\log_{10}$ shell dry weight were significantly different among months at site 3. Regression slopes varied seasonally; slopes were greatest in the winter, declined during the spring, and reached a minimum in August (Figure 2). Analysis of covariance indicated that slopes for regressions of $\log_{10}$ tissue weight on $\log_{10}$ length varied among months in a similar pattern (Table 2). Because the coefficients of determination for the regressions on shell length were somewhat greater than those for regressions on shell weight, all further analysis used regressions on shell length.

There were no significant differences between years in slopes of regressions for the same site; however, adjusted mean tissue weights were significantly different between years for three of the four sites (Table 3). For these three sites, adjusted tissue weights were greatest in 1994. There were no significant differences in regression slopes among sites each year; however, most within year comparisons of adjusted tissue weights between sites were significantly different (Table 4). Although there was no longitudinal trend, adjusted tissue weights were greatest for mussels collected from the more lentic sites (2, 3 and 4) each year.

Discussion

Under controlled conditions, determining the relationship between tissue weight and shell morphometrics can be effective for assessing short-term stress in unionids (Payne and Miller 1987). Comparisons of such relationships to evaluate stress of wild populations has had variable results. Houslet and Layzer (these proceedings)

found a substantial difference in adjusted tissue weights for mussels from a site impacted by coal mining compared to mussels from an unimpacted site. In contrast, Lewandowski (1976) did not find any difference between mean weights of unionids with and without zebra mussels attached. Similarly, Nalepa (1994) concluded that weight length relationships were not sensitive enough to measure stress in unionids infested with zebra mussels.

Our results indicate that the relationship between tissue weight and shell length is a very sensitive measure of mussel condition. Indeed, this relationship seems too sensitive to slight differences in environmental conditions or normal seasonal variation in mussel condition. For instance, regression slopes calculated for each monthly sample collected at site 3 appear to reflect the annual gametogenic cycle of *F. ebena* in Kentucky Lake (J. Heinricher, unpublished data). Failure to control for such normal seasonal variations in mussel condition could obscure any changes due to the presence of zebra mussels. Likewise, the significant differences among sites in weight-length regressions for *F. ebena* clearly indicate the need to evaluate mussel condition on a site-specific basis. Although we anticipated that the relationship between tissue weight and shell length might differ among sites due to possible differences in habitat quality or food abundance in the nearly 300-km-long study area, we did not design our study to test for differences in condition among samples collected over relatively short distances. Nonetheless, we recommend that composite samples of mussels collected from different locations not be used for monitoring condition unless justified by a priori determination of no intersite differences. Monitoring mussel condition to detect the effects of zebra mussels or other

perturbations can be controlled for variation due to location and season; however, the between year differences which occurred at most of our sites would be difficult to partition out.

An alternative method for evaluating the effects of zebra mussels on unionids is to monitor glycogen content, the primary energy store in mussels. However, the concentration of glycogen in unionids seems to vary in much the same way that tissue weights of *F. ebena* did in the Tennessee River. For instance, Jadhav and Lomte (1982) found that glycogen content varied seasonally and reflected the reproductive cycle of *Lamellidens corrianus*. In controlled experiments, Haag et al. (1993) monitored glycogen content of lampsilines and amblemines and were able to assess short-term effects induced by zebra mussels. Because glycogen content of mussels can be determined from a nonlethal mantle biopsy (Berg et al. 1995), its use could be advantageous compared to using total dry tissue weight to monitor mussel condition. Although Haag et al. (1993) were able to detect significant differences in glycogen content of lampsilines and anodontines from natural populations in Lake Erie, glycogen content was not correlated with zebra mussel infestations on amblemines in Lake Erie. Since anodontines and lampsilines comprised a small portion (<10 %) of the mussel assemblage at our sites, it would have been difficult to obtain adequate sample sizes of these species for glycogen analysis, particularly if samples were selected for size or sex. Likewise, it might be difficult to obtain sufficient samples of anodontines and lampsilines from other big rivers because amblemines also dominate the present fauna in those river (Williams and Schuster 1989; Holland-Bartels 1990).

Regardless of whether glycogen content or tissue weight is used to monitor mussel condition, decisions on when to "rescue" unionids will likely be based on the best estimates of the probability of surviving in the wild compared to surviving in captivity. As yet, there are no guidelines for making these estimates; long-term studies relating survival to initial mussel condition should be initiated to establish threshold levels of mussel condition beyond which, rescue attempts would be futile. Since densities of zebra mussels have remained low at our sampling sites, we are cautiously optimistic that such thresholds will not be needed for the mussel fauna in the Tennessee River.

Acknowledgements

Funds for this study were provided by the Kentucky Department of Fish and Wildlife Resources, the Tennessee Wildlife Resources Agency, the U. S. Fish and Wildlife Service, and the Center for the Management, Utilization, and Protection of Water Resources at Tennessee Technological University. We thank T. G. Cochran, M. A. Hartman, J. R. Heinricher, D. Hubbs, and M. A. Pegg for their assistance in collecting samples.

Literature Cited

Berg, D.J., W.R. Haag, S.I. Guttman, and J.B. Sickel. 1995. Mantle biopsy: a technique for nondestructive tissue-sampling of freshwater mussels. Journal of the North American Benthological Society 14:577-581.

Dunn, C.S., and J.B. Layzer. . Evaluation of various facilities for maintaining freshwater mussels in captivity. Pages *in*: , eds. Conservation and Management of Freshwater Mussels II Initiatives for the Future. Proceedings of a UMRCC Symposium, 16-18 October 1995, St. Louis, Missouri. Upper Mississippi River Conservation Committee, Rock Island, IL.

Gillis, P.L., and G.L. Mackie. 1994. Impact of the zebra mussel, *Dreissena polymorpha*, on populations of Unionidae (Bivalvia) in Lake St. Clair. Canadian Journal of Zoology 72:1260-1271.

Griffiths, R.W., D.W. Schloesser, J.H. Leach, and W.P. Kovalak. 1991. Distribution and dispersal of the zebra mussel (*Dreissena polymorpha*) in the Great Lakes Region. Canadian Journal of Fisheries and Aquatic Sciences 48:1381-1388.

Haag, W.R., D.J. Berg, D.W. Garton, and J.L. Farris. 1993. Reduced survival and fitness in native bivalves in response to fouling by the introduced zebra mussel (*Dreissena polymorpha*) in western Lake Erie. Canadian Journal of Fisheries and Aquatic Sciences 50:13-19.

Hebert, P.D.N., B.W. Muncaster, and G.L. Mackie. 1989. Ecological and genetic studies on *Dreissena polymorpha* (Pallas): a new mollusc in the Great Lakes. Canadian Journal of Fisheries and Aquatic Sciences 46:1587-1591.

Holland-Bartels, L.E. 1990. Physical factors and their influence on the mussel fauna of a main channel border habitat of the upper Mississippi River. Journal of the North American Benthological Society 9:327-335.

Houslet, B.S., and J.B. Layzer. . Difference in growth between two populations of *Villosa taeniata* in Horse Lick Creek, Kentucky. Pages in: , eds. Conservation and Management of Freshwater Mussels II Initiatives for the Future. Proceedings of a UMRCC Symposium, 16-18 October 1995, St. Louis, Missouri. Upper Mississippi River Conservation Committee, Rock Island, IL.

Jadhav, M.L., and V.S. Lomte. 1982. Seasonal variation in biochemical composition of the freshwater bivalve, *Lamellidens corrianus*. Rivista di Idrobiologia 21:27-36.

Lewandowski, K. 1976. Unionidae as a substratum for *Dreissena polymorpha* Pall. Polskie Archiwum Hydrobiologii 23:409-420.

Mackie, G.L. 1991. Biology of the exotic zebra mussel, *Dreissena polymorpha*, in relation to native bivalves and its potential impact in Lake St. Clair. Hydrobiologia 219:251-268.

Naimo, T., P. Thiel, and K. Welke. . An evaluation of relocation of unionid mussels into an artificial pond. Pages *in*: , eds. Conservation and Management of Freshwater Mussels II Initiatives for the Future. Proceedings of a UMRCC Symposium, 16-18 October 1995, St. Louis, Missouri. Upper Mississippi River Conservation Committee, Rock Island, IL.

Nalepa, T.F. 1994. Decline of native unionid bivalves in Lake St. Clair after infestation by the zebra mussel, *Dreissena polymorpha*. Canadian Journal of Fisheries and Aquatic Sciences 51:2227-2233.

Payne, B.S., and A.C. Miller. 1987. Effects of current velocity on the freshwater bivalve *Fusconaia ebena*. American Malacological Bulletin 5:177-179.

Payne, B.S., and A.C. Miller. 1989. Growth and survival of recent recruits to a population of *Fusconaia ebena* (Bivalvia: Unionidae) in the lower Ohio River. American Midland Naturalist 121:99-104.

Schloesser, D.W., and T.F. Nalepa. 1994. Dramatic decline of unionid bivalves in offshore waters of western Lake Erie after infestation by the zebra mussel, *Dreissena polymorpha*. Canadian Journal of Fisheries and Aquatic Sciences 51:2234-2242.

Steele, R.G.D., and J.H. Torrie. 1980. Principles and procedures of statistics a biometrical approach. 2nd edition. McGraw-Hill Book Company, New York, New York.

Todd, R.M. 1993. Tennessee's commercial musseling regulations. Pages 32-37 *in*: K.S. Cummings, A.C. Buchanan, and L.M. Koch, eds. Conservation and Management of Freshwater Mussels. Proceedings of a UMRCC Symposium, 12-14 October 1992, St. Louis, Missouri. Upper Mississippi River Conservation Committee, Rock Island, IL.

Williams, J.C., and G.A. Schuster. 1989. Freshwater mussel investigations of the Ohio River mile 317.0 to mile 981.0. Kentucky Department of Fish and Wildlife Resources, Frankfort, KY. 57pp.

Table 1. Numbers and species of unionids collected in quantitative samples at four sites on the Tennessee River in 1993.

Species	Site 1	2	4	5
Amblema plicata	24	19	3	2
Arcidens confragosus	1	--	--	--
Cyclonaias tuberculata	6	--	--	5
Ellipsaria lineolata	11	--	--	8
Elliptio crassidens	3	--	--	1
Elliptio dilatata	7	--	--	--
Fusconaia ebena	78	11	28	216
Fusconaia flava	4	5	1	--
Lasmigona complanata	1	--	--	--
Leptodea fragilis	9	1	--	6
Megalonaias nervosa	5	1	3	--
Obliquaria reflexa	10	10	15	25
Pleurobema cordatum	4	--	--	1
Potamilus alatus	5	9	6	1
Quadrula metanevra	1	--	--	5
Quadrula pustulosa	73	--	2	39
Quadrula quadrula	18	2	10	4
Truncilla donaciformis	7	--	29	37
Truncilla truncata	9	--	--	--
Unidentified juveniles	32	1	19	3
Total	308	59	116	353
Density (#/m^2)	61.6	11.8	23.2	70.6

Table 2. Least squares regressions of LOG_{10} tissue dry weight (TDW) on LOG_{10} length (L) of *Fusconaia ebena* collected at site 3 in Kentucky Lake between November 1993 and October 1994. $LOG_{10}TDW = LOG_{10}b_0 + b_1LOG_{10}L$.

Month	b_0	b_1	R^2	N	Mean Length ± SD (mm)
January	-5.570	3.354	.97	50	56.5 ± 13.7
February	-5.210	3.175	.96	57	55.8 ± 11.2
March	-4.918	2.996	.95	50	56.4 ± 11.4
April	-5.111	3.074	.97	56	60.7 ± 11.7
May	-4.672	2.889	.96	48	63.9 ± 10.2
June	-4.626	2.856	.97	55	56.7 ± 10.5
July	-4.747	2.911	.94	52	57.9 ± 7.9
August	-4.279	2.639	.91	50	56.4 ± 7.6
September	-4.906	3.019	.95	53	56.4 ± 8.8
October	-4.704	2.879	.94	55	59.3 ± 10.5
November	-5.317	3.198	.98	56	56.7 ± 15.9
December	-5.190	3.111	.97	51	59.4 ± 14.6

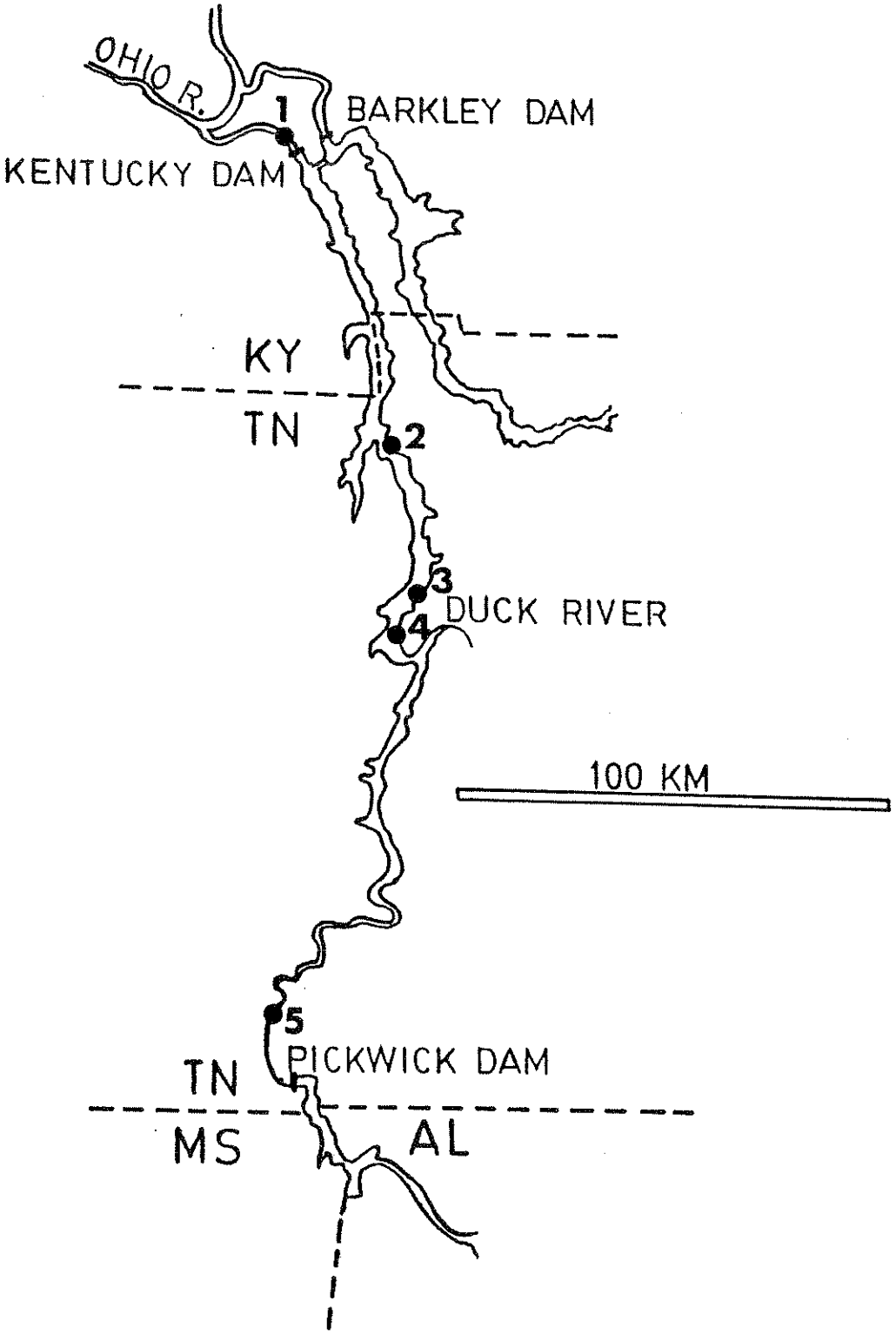
Table 3. Comparisons of adjusted mean dry tissue weights between years for each site (n.s. = not significant).

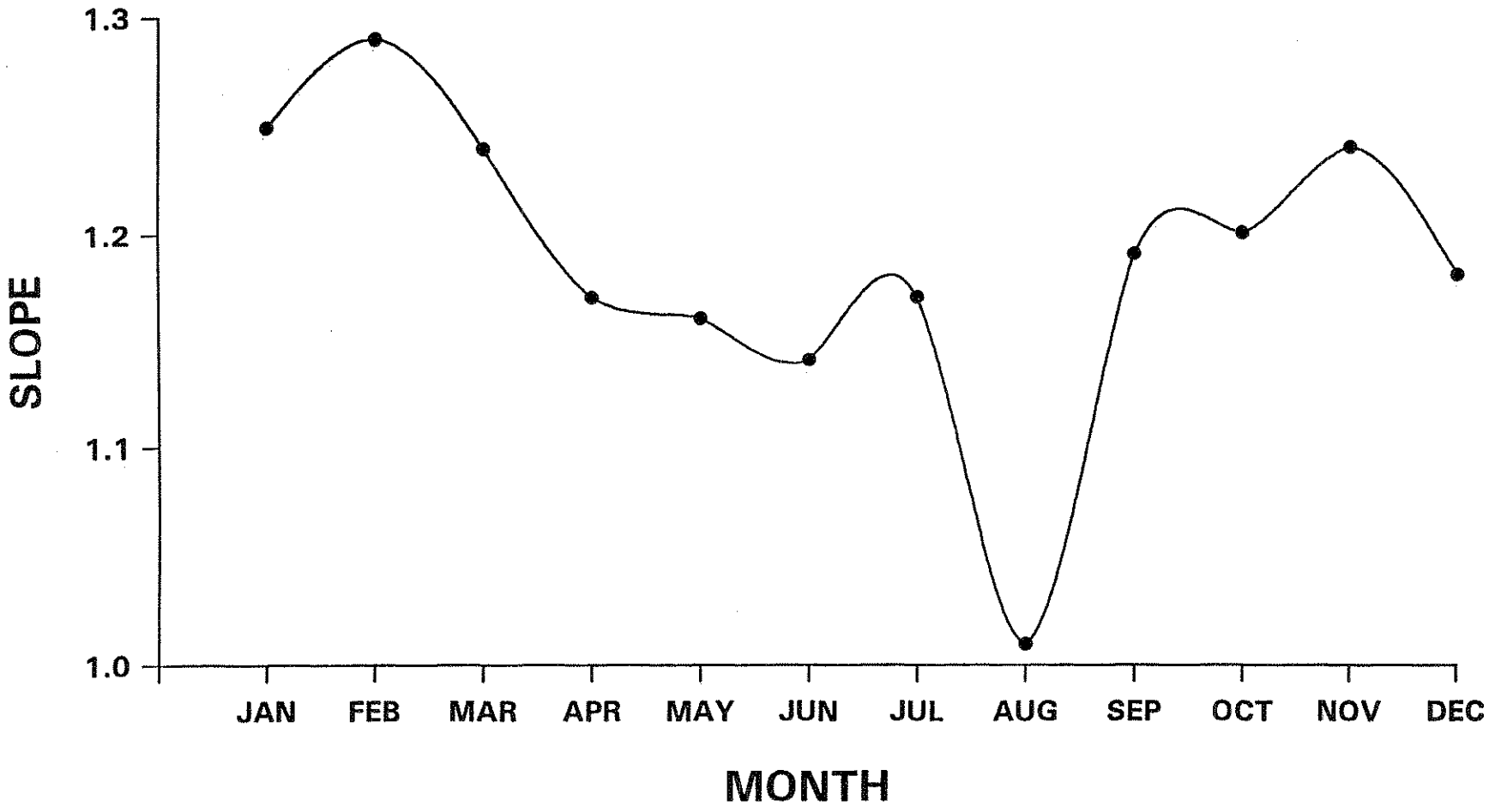
	Mean Tissue Weight (g)			
	1993	1994	P	Mean Shell Length (mm)
Site				
1	2.331	2.498	0.0197	63
2	4.306	4.823	0.0004	72
4	1.778	1.963	0.0003	54
5	1.848	1.779	n.s.	56

Table 4. Comparisons of mean dry tissue weights adjusted for shell length (1993: 61 mm; 1994: 60 mm) among sites. (n.s. = not significant; * = $P < 0.01$; ** = $P < 0.001$; *** = $P \leq 0.0001$, for pair-wise site comparisons within each year).

			Site Compared With			
Site	Year	Mean Tissue Weight (g)	2	3	4	5
1	1993	2.108	***	---	***	**
	1994	2.124	***	***	***	n.s.
2	1993	2.697		---	**	***
	1994	2.840		**	*	***
3	1993	---			---	---
	1994	2.589			n.s.	***
4	1993	2.426				n.s.
	1994	2.584				***
5	1993	2.346				
	1994	2.171				

List Of Figures


OHIO R.
1
BARKLEY DAM
KENTUCKY DAM
KY
TN
2
3
DUCK RIVER
4
100 KM
5
PICKWICK DAM
TN
MS
AL


1.3
1.2
1.1
1.0
SLOPE
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC
MONTH