AN INVESTIGATION OF THE POTENTIAL OF FRESHWATER MUSSELS AS SEASONAL INDICATORS IN ARCHAEOLOGICAL SITES

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An Investigation of the Potential of Freshwater Mussels as Seasonal Indicators in Archaeological Sites

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ABSTRACT

Evidence has been presented in the palaeontological literature that marine bivalves preserve in their calcareous shells a record of the environmental events and astronomical periodicities which affect their metabolic processes. The most prominent feature of this record is the external growth ring initiated by the annual decline in the mean temperature of the environment. It has been shown that small scale events based on circadian and tidal rhythms are also recorded. Recent articles in archaeological journals have suggested that this information forms the basis for estimating the season of occupation of many archaeological sites.

In order to evaluate the potential of freshwater mussels as seasonal indicators in archaeological sites, a modern sample of live mussels was sequentially gathered in the Canadian Interior Basin, together with pertinent environmental information. The sample was analysed using a variety of the methods which have been proposed in the literature. To evaluate macroscopic techniques of estimating seasonality, a random sample of Lampsilis siliquoidea valves was drawn from the study population. Using this sample, a series of independent observer tests were made which indicated that macroscopic techniques of analysis for the estimation of seasonality were highly unreliable. To evaluate microscopic techniques, radial shell

sections were prepared as acetate peel replicas and thin sections for microstructural analysis by means of the scanning electron microscope (SEM) and a binocular light microscope. Stained thin sections, examined with transmitted light, were found to be the most satisfactory technique from the standpoints of consistent results, ease of study, and maximum yield of information.

While the variability of the specimens indicated that a moderately large sample is required, the results of the thin section analysis indicated that seasonality estimates may be derived with reasonable accuracy from the shells of freshwater mussels. The season in which the mussels died may be estimated by means of averages calculated from the counts of microincrements contained in the final macroincrement deposited on the ventral edge of the shells in a sample. The results of this research confirm the hypothesis that the patterns of shell deposition in freshwater mussels are principally the result of metabolic reactions to environmental variables and form a reliable basis for seasonality estimates.

This thesis is

gratefully dedicated to

LYDIA NICHOLSON and LOUIS SORENSEN

without whose support it would

never have been written.

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I must extend my thanks to the many fellow students who assisted me in my research. Special thanks is due to Gerry Oetelaar and Jean Williams who read and commented upon an early draft of this thesis. I would also like to express my appreciation to Ann Thompson who gave me many hours of her time as a voluntary research assistant, supervising observer tests and counting microincrements with the light microscope.

I am indebted to Dr. Leigh Syms of the Sociology/Anthropology Department and Dr. Robert Springer of the Geology Department at Brandon University for making laboratory space and thin sectioning equipment available to me during the summer of 1979, when I was gathering my live samples in Manitoba. I

would also like to thank Dr. Robert Hamilton of the Canada Department of Agriculture Research Station who obtained minimum and maximum air temperature readings from the station for my research.

The author owes a special debt of gratitude to former Brandon University classmate Scott Hamilton who continued to collect and forward specimens from the confinement cages, and from the river bottom after the author had returned to Simon Fraser University in the fall, when the river was covered with a thick layer of ice. Finally, I would like to thank my wife,Lydia, who did most of the typing of the several drafts of this thesis.

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INTRODUCTION

Evidence has been presented in the palaeontological literature that marine bivalves preserve in their calcareous shells a record of the environmental events and astronomical periodicities which affect their metabolic processes (Andrews 1972; Barker 1964,1970; Clark 1968,1974b,1975; Cunliffe 1974; Cunliffe and Kennish 1974; Evans 1972,1975; Farrow 1971; Hall et al. 1974; Hall 1975; Kennish and Olsson 1975; Pannella and MacClintock 1968a,1968b; Pannella 1975; Rhoads and Pannella 1970; Richarson et al. 1979; Runcorn 1968; Thompson 1975; Whyte 1975). The most prominent feature of this record is the external growth ring initiated by the annual decline in the mean temperature of the environment. It has been shown that small scale events based on circadian and tidal rhythms are also recorded.

Recent articles in archaeological journals have suggested that this information forms the basis for estimating the season of occupation of many archaeological sites (Coutts and Higham 1971; Ham and Irvine 1975; Koike 1973,1975,1979; Ray 1976; Shaw 1978). The author has noted the presence of freshwater mussel shell in many prehistoric sites in the Canadian Interior Basin. To evaluate the potential of these freshwater mussel species as seasonal indicators, a live sample was collected in this region together with pertinent environmental information. Analysis of this sample generated the data base for this thesis.

In order to understand the mechanics and theory of growth line formation in freshwater mussels, this thesis has drawn heavily on research done on marine species. The reason for this is that there is virtually no literature dealing with the infrastructure and microstructure of freshwater bivalves. There is, however, extensive literature on marine bivalves, and preliminary research has indicated that the recognizable features of freshwater shells are essentially similar to those of many marine species.

In the area covered by this research, only two families of bivalves are represented: the Unionidae and the Sphaeriidae. There is no evidence to suggest that Sphaeriidae, which are usually less than one cm in diameter were of any economic importance to aboriginal peoples in this area. There are 13 species of Unionidae in the research area (Clarke 1973); eight of these species are represented in the present research sample. The other five species are structurally similar to those recovered in the research sample. The most frequently occuring species is Lampsilis siliquoidea which accounts for 70 percent of the total sample.

It is the intention in this thesis to critically examine methods which have been proposed in the literature for determining seasonality from bivalve shells. The potential of these methods, for accurately determining the season during which a sample of freshwater mussels was collected, is evaluated and the conclusions are presented. The investigation of macroscopic and microscopic growth structures, which were

observed in a sample of mussel shells collected from the Canadian Interior Basin, is outlined, and directions for further research suggested.

The thesis is composed of five major sections. The first section outlines the design parameters, the field conditions underwhich the sample was collected and describes the field methods which were employed. The second section discusses the historical background of growth line research in bivalves and some of the attempts which have been made to interpret seasonality from bivalve shells. The third section describes the laboratory procedures and techniques employed to prepare the shells for study. Section four describes the microscopic examination of the infrastructure of L. siliquoidea and outlines the results of this investigation. Finally, section five offers a tentative evaluation of the potential of the other species in the sample.

Chapter 1: DATA COLLECTION

In an attempt to understand the pattern of deposition of growth structures in freshwater mussels and to select species most likely to occur in archaeological sites, seven guidelines were established which served as parameters for the collection of the mussels whose shells were to serve as the data base for the research underlying this thesis. These guidelines were as follows:

1. The sample must be collected live and the date of death recorded.
2. The sample must be collected from more than one location to evaluate the importance of localized environmental effects, and to minimize the chance of sample error due to such local effects.
3. The sample must be collected over an extended period of time from the same stations to facilitate the evaluation of the relationship between shell increments and astronomical, biological and other environmental periodicities.
4. A record of air and water temperatures affecting the environment of the mussels must be kept.
5. A species should be selected in which the sex of the animal can be readily distinguished on the basis of shell morphology to facilitate the assessment of sex related biological rhythms.

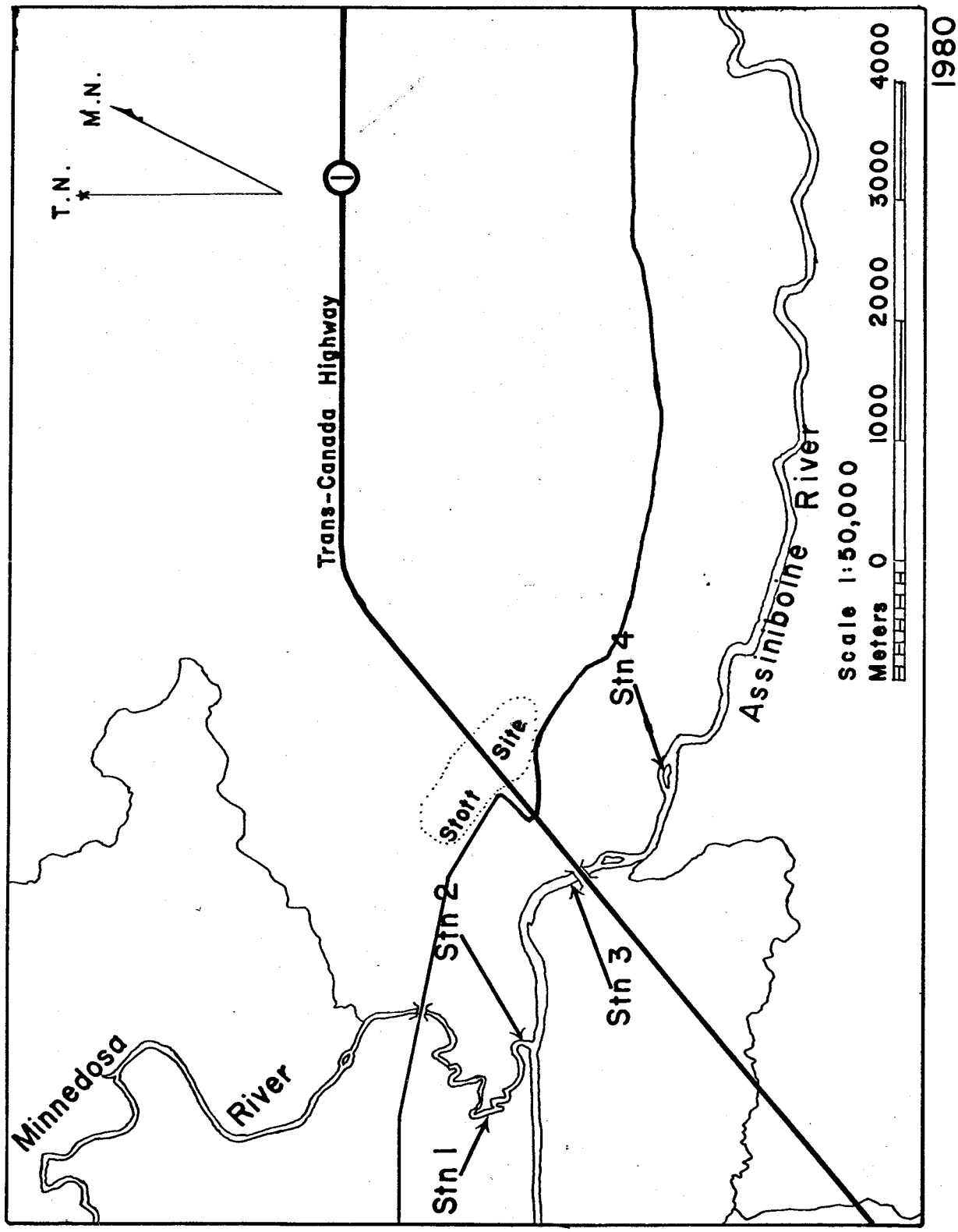
6. A relatively abundant shallow water species will have the greatest chance of being included in an archaeological assemblage.

7. The sample should be gathered by means of a technology readily available to primitive man in the area under study.

The samples which form the data base for this thesis were collected during the summer of 1979 from four stations located in the Minnedosa and Assiniboine rivers (Figure 1). Collection of mussels did not commence until July 8 because of unusually high water levels in the rivers. Snow from the extremely severe winter of 1978/1979 remained in sheltered locations until the first week of June in the study area. As a result of this late melt of the snow, the spring run-off from the Riding Mountain uplands into the Minnedosa River did not abate until the last week in June. In the early spring, in an attempt to mitigate flooding in the Red River Valley, water at the headwaters of the Assiniboine River was impounded to a much greater depth than usual behind the Shellmouth Dam. After the floodwaters had receded in the Red River Valley, this water was released into the Assiniboine channel creating artificially high water levels until midsummer. During the month of August the level of the Assiniboine dropped rapidly when the flow from the Shellmouth Reservoir was suddenly restricted in response to a summer of extremely low rainfall.

The Minnedosa River is a small rapidly flowing stream which has its origin in the Riding Mountains, a high remnant of Cretaceous shale rising 300 meters above the surrounding prairie.

Figure I. Map of Research Area (Ref. 62 J/5)



The Minnedosa stream bed, at its lower end, is approximately 30 meters wide but during dry summers the stream itself may dwindle to a width of four or five meters. The stream bed is composed of sand and gravel, and large boulders are scattered through the stream bed and along the banks.

Collecting station number one was located approximately one kilometer upstream from the junction of the Minnedosa River with the larger Assiniboine River (Figure 1). The eastern margin of the channel at this point was composed of a large gravel bar which gradually merged into a sandy bottom sloping towards a cut bank on the western edge. All mussels recovered at this station were found on the sandy bottom in less than one half meter of water.

Collecting station number two was located approximately 100 meters above the junction of the Minnedosa River with the Assiniboine River. The stream bed and channel morphology were the same as at station one, except that the gravel bar and the cut bank were reversed due to the angle at which the channel directed the flow of water. All mussels at this station were collected from a sandy bottom in less than one half meter of water.

The Assiniboine River is a medium sized prairie river of approximately 60 meters in width. It is an underfit stream which meanders through a two kilometer wide floodplain. This floodplain is an old glacial spilway which once drained meltwater from what is now central Saskatchewan into glacial Lake Agassiz. The cut banks of the present channel are composed entirely of silt and

the streambed is a mixture of sand and silt interspersed with cobbles and occasional lenses of gravel mixed with silt.

Collecting station number three was located 20 meters upstream from the bridge where the Trans-Canada Highway crosses the Assiniboine River. The mussel bed at this station was located on the south bank of the river on a silt substrate. All mussels recovered from this station were collected in less than one half meter of water.

Collecting station number four was located approximately one and one half kilometers downstream from the Trans-Canada bridge. All the mussels at this station were collected in less than one half meter of water from a gravel and silt substrate at the upstream end of an island in the center of the river.

Samples were collected at these sites on July 8, July 23, August 8, August 17, August 29, September 28 and November 26. In all cases the mussels were killed by immersion in boiling water within 12 hours of collection. In Table 1 it can be seen that the sexually dimorphic species, Lampsilis siliquoidea, was present at all collection sites. It was also present in all samples collected. This species represents 70 percent of the total sample, and in view of the fact that a conscious effort was made at station four to collect the less abundant species, after a small number of L. siliquoidea specimens had been gathered, L. siliquoidea comprises a much higher percentage of of the present river population than these figures would indicate. It is noteworthy that at stations one, two and three, where the mussels were gathered without regard to species,

Table 1. Frequency of Species by Station

Species	Station One	Station Two	Station Three	Station Four	Species Total
L. siliquoidea	33	14	107	21	175
L. ovata	11	5	2	19	37
Ligumia recta	1		2	11	14
Lasmigona complanata	2		2	5	9
Amblema plicata				7	7
Anodonta grandis	1		3	1	5
Fusconaia flava				2	2
Strophitus undulatus				1	1
Station Total	48	19	116	67	250

Lampsilis ovata had a much higher frequency, relative to L. siliquoidea, at stations one and two in the Minnedosa River than at station three in the Assiniboine River (Table 1).

In addition to collecting mussels directly from the river bottom at the four stations, an attempt was made to establish artificial landmarks in several of the shells. By introducing these marks at a known point in time, it would be possible to relate all subsequent depositional patterns to a known event. This method would give more precise information on the timing and frequency of growth structures observed in the infrastructure of the valves. Work done by marine researchers (Hidu and Hanks 1968; Koike 1973) has indicated that when specimens of Mercenaria mercenaria and Meretrix lusoria were immersed in a seawater solution containing Alizarin Red S, they deposited a band of coloured shell. This readily observed band served as a temporal marker within the infrastructure of the shell. These experiments indicated that there was a minimal disruption of the metabolic processes of the bivalves, and patterns of deposition returned to normal within one to three days (Hidu and Hanks 1968). Other methods of marking, such as the use of waterproof ink, have been found to be unreliable due to their susceptibility to removal by abrasion. The ink method has also been found to traumatize the animals and introduce a hiatus in growth and, in consequence, a major growth line. The shell must be air dried before the ink is applied, and the ink must be allowed to dry before the shell can be returned to the water (Barker 1970:79). This period of dessication initiates an extended period of recovery during

which no shell is deposited. Shells can be marked by notching, but studies indicate that there is a significant hiatus in the deposition of shell until the animal recovers from the shock and repairs the damage (Hidu and Hanks 1968; Koike 1973).

On July 12, 68 mussels were collected and placed in a 20 ppm Alizarin Red S solution for periods of one hour and two and one half hours. These stained mussels were placed in wire cages measuring 45x50x25 cm. The cages were located at stations one, two and three. A fourth cage, with a wire divider, containing stained mussels from both the Minnedosa and Assiniboine rivers, was located in a beaver pond in a small, spring fed creek on 17-11-13 W1 (Table 2).

The cages were revisited on July 21, and samples were collected, killed and examined to verify staining. The divided cage, with the relocated mussels, was found to be above water due to a failure of the beaver dam. All 10 of the mussels from the Assiniboine River had died. All of the mussels from thr Minnedosa River were still alive, although three died shortly thereafter. All specimens were L. siliquoidea. The reason for the differential survival of the two groups is unknown. None of the mussels had deposited coloured shell, and there was no evidence of staining of the flesh.

On July 23, all mussels were removed from the cages and placed in a 20 ppm Alizarin Red S solution of river water and held there for 60 hours. The mussels were returned to their cages and left undisturbed until August seventh. At this time, it was found that the cage at station one had recently been

Table 2. Caged and marked mussels showing date and numbers of mussels set out in cages, and the numbers found dead in the cages or collected live from the cages.

Date	Cage One			Cage Two			Cage Three			Cage Four			Cage Five		
	S.O.*	D.*	C.L.*	S.O.	D.	C.L.	S.O.	D.	C.L.	S.O.	D.	C.L.	S.O.	D.	C.L.
July 12	14	--	--	16	--	--	20	--	--	--	--	--	--	--	--
13	--	--	--	--	--	--	--	--	--	18	--	--	--	--	--
21	--	--	2	--	--	2	--	--	2	--	--	2	--	--	--
23	Restained			Restained			Restained								
26	--	--	--	--	--	--	--	--	--	Rest. 7			--	--	--
29	--	--	--	--	--	--	--	--	--	10	6	--	--	--	--
Aug. 7	--	3	--	--	--	--	--	--	--	--	--	--	22	--	--
28	--	--	4	--	7	7	--	--	6	--	1	12	--	--	10
Nov. 9	--	1	4	--	--	--	--	1	11	--	--	--	--	--	12
Totals	14	4	10	16	7	9	20	1	19	28	14	14	22	--	22

* S.O. - Set Out D. - Died C.L. - Collected Live

pulled from the water by persons unknown and discarded on the bank. Three of the mussels in the cage were dead. The seven remaining live mussels were transported in a plastic bucket of river water and relocated in their cage at station three in the Assiniboine River. The cage at station two was partially buried by a moving sand bar and had to be relocated in deeper water. The restriction of the flow of water from the Shellmouth Reservoir had resulted in a rapid lowering of the water level in the Assiniboine. This lowering of the water level exposed the upper portion of cage number three. However, the mussels were still submerged and the cage was simply placed more deeply in the water. At this time an additional cage was located at station three, and 22 mussels were collected and marked with a file on their ventral edges. These file marked mussels were placed in this fifth cage.

On August 29, samples were collected and killed from all cages. The mussels appeared to be healthy and it was noted that the marsupial gills of the females were swollen with glochidia. The flesh of the mussels was stained pink from the Alizarin Red S solution, but there was no evidence of the deposition of a stained layer of shell. Marine researchers (Hidu and Hanks 1968; Koike 1973) have indicated that stained specimens of Mercenaria mercenaria and Meretrix lusoria rapidly deposit coloured bands within the infrastructure of their shells and within a few days the animals have expelled all Alizarin Red S dye from their flesh. The L. siliquoidea in this experiment had neither deposited coloured shell nor eliminated the dye after 36 days.

On November 9, a colleague, Scott Hamilton, collected and killed the remaining mussels from all the cages. At this time there were approximately 5 cm of ice on the river which had to be broken to reach the cages. On this occasion, and again on November 26 when a sample was collected from the river bed at station three, a wet suit was used to enter the water.

None of the mussels which were exposed to the Alizarin Red S solution deposited an observable coloured band in their shell structure. Although the mussels exposed to the 20 ppm concentration of Alizarin Red S dye retained the dye in their flesh for over 36 days, and although several specimens had resumed growth when the final sample was collected from the cages on November 9, the only traces of Alizarin Red S dye which were observed in this sample were found in the periostracal layer. This staining appeared to result from the immersion of the shells in the Alizarin Red S solution, rather than from deposition by the mantle.

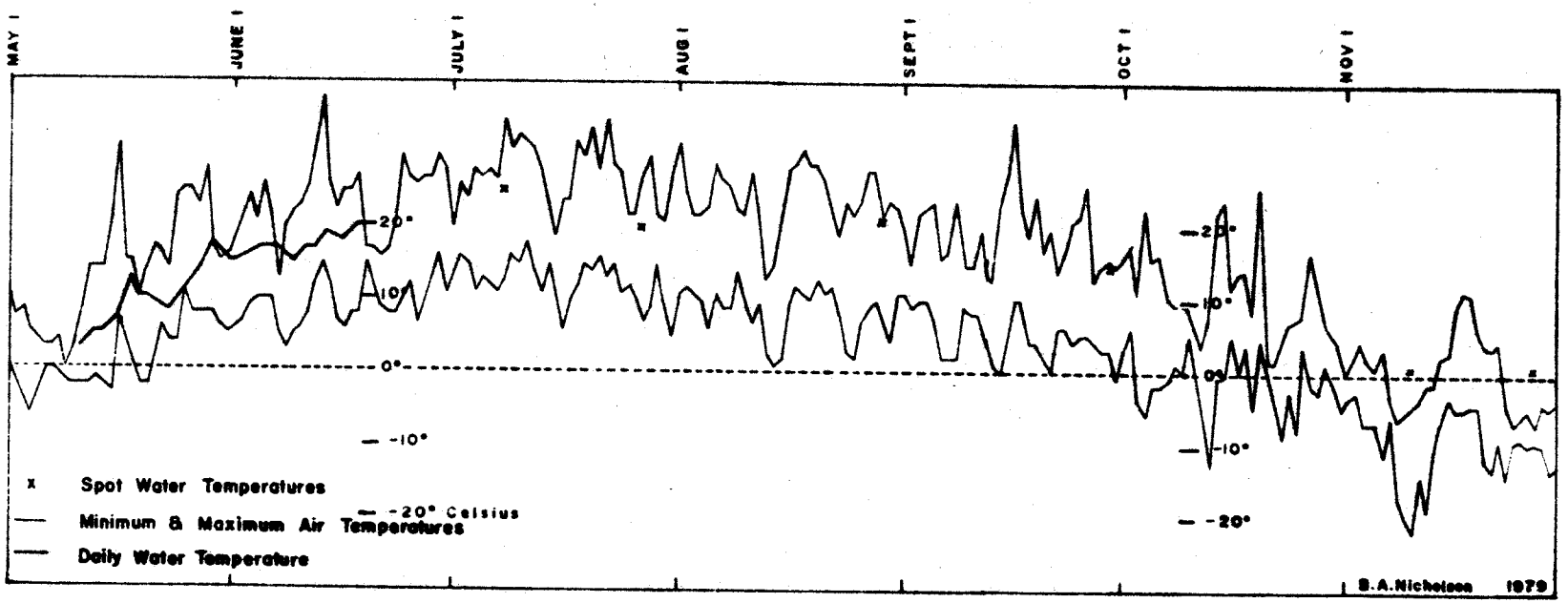
The mussels which had been marked with a file on their ventral margins resumed growth earlier than did those exposed to the Alizarin Red S solution. However, a hiatus of at least 50 days seems to be indicated for these specimens as well. The traumatic effect of the marking procedures upon the mussels, together with the difficulties experienced with lowered water levels and the removal of one of the cages from the Minnedosa River by persons unknown, largely destroyed the usefulness of the caged specimens. In the final analysis no significant information on growth periodicities could be obtained from the

marking experiments.

The samples which were collected from the river bed and immediately killed form the useable portion of the data base. The most constant environmental conditions in terms of a uniform substrate and a continuous, uniform water flow were found at station three. Lampsilis siliquoidea specimens were collected at intervals from a single location at this station over a period of 141 days, ending November 26. Between May 10 and June 16 the temperature of the Assiniboine River rose from 3 degrees Celsius to 20 degrees Celsius (Figure 2). For the balance of the summer and fall, only spot checks were taken infrequently. Minimum and maximum air temperatures were obtained from the Agriculture Canada Research Station at Brandon. These temperatures were periodically checked against mid-afternoon temperatures recorded in the shade at the various stations and found to coincide within one degree on the occasions when such comparisons were made.

The sample which was collected from these stations reflects the unique conditions of growth experienced during the spring of 1979. In most years, mussels collected on July 8 would be expected to show a significant amount of growth in the latest macroincrement along their ventral edges. However, in the spring of 1979, water temperaturesdid not rise above 10 degrees Celsius for more than a couple of days at a time until the final week of May (Figure 2). It is unlikely that the mussels began to secrete shell before the first week of June. The small increments which were observed in the July 8 sample indicate that they were

Figure 2. Air and Water Temperatures in the Brandon Area - Summer 1979



harvested early in the season: a conclusion consistent with the late spring experienced in 1979.

The sequentially collected sample of 48 mussels from station three comprised the only assemblage which could be assumed to demonstrate continuous deposition of shell increments between spring break-up and the times of collection. Members of all the other assemblages experienced trauma introduced by experimental procedures and fluctuating water levels, or represented collections over short time periods. The sample collected at station three represented controlled recovery over a period of 141 days of a single, sexually dimorphic species, L. siliquoidea.

Chapter 2: HISTORY OF GROWTH LINE RESEARCH AND SEASONALITY STUDIES

For a very long time, it has been known that the concentric rings on marine and freshwater shells record the passage of discrete units of time. Barker (1970:2) notes that Leonardo da Vinci believed that there was "... a relationship between the growth layers of invertebrate exoskeletons and lunar monthly and annual cycles."

Work done by fisheries researchers interested in population studies have concentrated on the macroscopically observed annuli and on absolute size. An example is the report of Stevenson and Dickie (1954) based on collections of Placopecten magellicanus from the Bay of Fundy. The report concludes, "The results show that the growth rings are annual rings formed during the winter months when water temperatures are at a minimum for the year"(Stevenson and Dickie 1954:661).

More recently studies have concentrated on the finer growth structures found in the infrastructure of the shells of a variety of bivalves. Palaeontologists and archaeologists have been inspired by Wells' (1963:948) observations on the incremental structures of Palaeozoic corals and his optimistic statement, "... every palaeontologist a geochronometrist; every fossil a geochronometer!" The assumptions of early microgrowth researchers were quite straightforward. Neville (1967:421)

stated that,

> "Daily growth layers are found in the structural parts of several biological systems. They provide a convenient experimental tool for researchers in several disciplines. They are a reflection of rhythmical metabolism which is synchronized with the astronomical environment."

Pannella and MacClintock (1968b:792) were more cautious in their approach, stating,

> "Circadian rhythms regulate almost universally the world of living matter, and it is reasonable to think they may leave a record in the skeletons of continuously growing organisms. ... From experiments in bivalve molluscs, it is safe to conclude that solar time is the basic unit reflected in the increments and that synodical time, at least in intertidal and shallow subtidal bivalves, is expressed in the thickness of the increments. ... Generic, specific, and individual differences should be carefully weighted in interpreting the growth patterns. ... When dealing with fossils, however, difficulties arise. Poor preservation, ambiguity of growth patterns, and lack of modern representatives make the counts of individual growth increments highly speculative."

A further note of caution was introduced by Cunliffe and Kennish (1974), who found a complex interplay of astronomical, physiological, and more immediate environmental forces in the patterning of growth increments. They state,

> "From what we have written so far, it should be clear that

> the shell of M. mercenaria is much like a tape recorder. Both the clam's general physiological condition and the many events in its environment are recorded in the pattern of daily increments. It should also be apparent that rings (breaks) on the surface of the clam shell record much more than the passing of years." (Cunliffe and Kennish 1974:24).

The need for detailed studies on live populations to determine the true nature of incremental structures in bivalves has been clearly demonstrated by Berta (1976), who compared a variety of macroscopic techniques of evaluating the age of Protothaca staminea with the microscopic examination of thin sections. Her work indicated that the results of the macroscopic studies did not correlate well with microscopic findings and that the macroscopic studies were highly inaccurate. Other studies of the microstructure of marine bivalve shells (Barker 1964,1970; Clark 1975; Crenshaw and Neff 1969; Evans 1972,1975; Hall 1975; Koike 1973; Kennish and Olsson 1975; Lutz and Rhoads 1977; Pannella and MacClintock 1968a; Thompson 1975; Whyte 1975) have shown that the deposition of growth increments is influenced by astronomical periodicities, latitude, temperature, salinity, food supply, endogenous physiological rhythms, species response and random events which disturb the metabolic processes of the animal.

Several researchers (Coutts and Higham1971; Ham and Irvine 1975; Ham 1976) have attempted to infer seasonality from marine shells by measuring the amount of growth following the last major growth ring. Recently it has been suggested that the same techniques be applied to archaeological sites containing the

shells of freshwater mussels (Ray 1976; Shaw 1978).

The usual method is to plot some dimension of growth such as length or width of the shell against the observed annuli or macroincrements on the surface of the bivalve shell. A growth curve can then be constructed, and the amount of expected seasonal growth for any age group of mussels can be predicted. The seasonality estimate is then based upon aging the mussel and measuring the amount of growth following the last major growth line. The degree to which the growth band is completed, or otherwise, is assumed to indicate the season in which the animal died.

There are a number of problems associated with this approach. The major one is the difficulty experienced in recognizing and defining criteria for distinguishing between growth lines which are seasonally induced annuli, and lines which are the result of hiatuses induced by spawning, storms, predator attacks and other environmental variables. Coutts and Higham (1971) simply discarded those shells in which they felt the distinction was not clear. They state:

> "The sorting of the main sample into classes according to the number of rings on the shell exterior may be complicated by the occurrence of disturbance rings. In many cases these could be distinguished from the main sequence of annual rings and ignored ... . If it proved impossible to distinguish between annual rings and disturbance rings, the specimens in question were rejected."(Coutts and Higham 1971:267).

They give no criteria for their acceptance or rejection of

growth rings.Consequently, their results are a reflection of a biased and idealized population rather than the real population. We do not know whether their study population represents 10 or 90 percent of the original collection from the beach. The sample selected for the construction of the growth curves consisted of discrete groups selected for well defined growth rings. "The individuals comprising the two subsamples from area A had either 4-5 or from 7-8 growth recession rings." (Coutts and Higham 1971:267). The usefulness of such a subjectively biased sample as a data base must be seriously questioned. The careful selection, for analysis, of samples of shells which conform to a predetermined pattern of deposition, with no attempt to account for the variabilitywhich has been selectively rejected, leaves the researcher with no clear picture of what his analysis really does represent.

A second major problem, associated with seasonality studies which are based on an assumption that the last growth line on a shell represents an annulus, is that individual bivalves and local populations may respond to some localized or unique event which bears little or no relationship to the cyclical variation in the inclination of the Earth's axis toward the sun. Barker (1970:215) states:

> "Observations of shell growth and of annual layer morphology make it evident that a regular winter hiatus is the rule for temperate species. This hiatus varies with individual organisms and environment and may or may not be preceded by a slow growth layer. It was also noted that

> pelecypods exposed to unfavourable environmental conditions may suspend the shell growth for days, weeks, months or as long as 1½ years at a time, or that this hiatus may be recorded as a disturbance ring only if shell growth is eventually resumed. Consequently, the lack of a slow growth deposit on the ventral margin of an empty shell is not a sure indication that the organism expired during the season of active growth, neither is the marginal slow growth layer of an empty shell necessarily an indication that fatality occurred in the slow growth season."

Each bivalve records the events of its own environment and physiology. Events such as predator attacks, parasites, disease or individual dislodgement, may induce a hiatus which is not representative of the population as a whole. This hiatus will define an increment which is not a complete annulus.

Berta (1976) has demonstrated that distinction between various types of growth lines cannot be made by means of macroscopic examination in the species Protothaca staminea. Kennish and Olsson (1975) found that, given adequate environmental data, various types of growth lines in Mercenaria could be related to specific environmental and physiological events such as temperature fluctuations, spawning and storms. Cunliffe (1974), who also studied Mercenaria, was less confident stating: "Storm breaks can be recognized with assurance only when they contain silt particles trapped deep inside surface indentations; neap tide breaks are rare and usually occur in winter or old age." Cunliffe (1974) also notes that growth increment patterns are species specific.

Most seasonality studies in which archaeologists (Coutts 1970,1975; Coutts and Higham 1971; Ham and Irvine 1975; Ham 1976) have attempted to distinguish between various types of growth lines do not give any of the criteria by which they make their distinctions. These discussions have tended to imply that the distinctions were universal and obvious. The extensive palaeontological literature (Barker 1964,1970; Berta 1976; Berry and Barker 1975; Clark 1968, 1974a,1974b,1975; Cunliffe 1974; Cunliffe and Kennish 1974; Dexter 1977; Evans 1972,1975; Farrow 1971; Hall 1975; Hall etal. 1974; House and Farrow 1968; Koike 1973, 1975, 1979; Kennish and Olsson 1975; Lutz and Rhoads 1977; Pannella and MacClintock 1968a,1968b; Pannella 1975; Rhoads and Pannella 1970; Thompson 1975; Whyte 1975) indicates clearly that the distinctions are neither universal nor obvious. The present research supports this more pessimistic view and suggests that often such criteria are subjectively and intuitively developed. Common sense suggests that criteria which cannot be described, and conclusions which are based on intuitively defined phenomena are of little value in serious research, even if they turn out to be correct.

Researchers from a wide range of disciplines have uncritically accepted the assumption that the growth rings on the surface of freshwater mussel shells represent annuli. Biologists (Chamberlain 1930; Coon etal. 1977; Hendelberg 1960; Kessler and Miller 1978; Stober 1972) have not generally questioned this assumption seriously. Similarily, archaeologists have taken this undemonstrated assumption to be true and have advocated seasonality studies accordingly (Ray 1976; Shaw 1978).

However, there is no theoretical reason to assume that factors of environment or physiology which disrupt the deposition of shell material in marine species would not similarily disrupt the deposition of shell material in freshwater mussels. Frost and thermal shocks (Kennish and Olsson 1975), which have been observed in tidal estuaries, are to be expected in shallow freshwater bodies. Sudden and often violent changes in water regime (often very localized in scope) due to the release of impounded water or runoff from a thunderstorm might well prove analogous to the effects of storms in a marine environment. An unsuccessful attack by a predator such as a raccoon, or the attachment of leeches, might affect shell deposition in freshwater mussels in a manner similar to that noted by Barker (1970) for gastropod attacks on marine species. Fungal infections (Gale 1977:11) or other diseases might also radically alter rates and patterns of shell deposition. Although there seems to be little reason to doubt that many of the growth rings represent the period when freshwater bodies in the Canadian Interior Basin are covered by as much as one half meter of ice, it is highly probable that some of the growth rings represent hiatuses induced by other causes.

To evaluate the reliability of macroscopic observation on the sample used in this research, two tests were run on 60 randomly selected L. siliquoidea shells drawn from a population of 175 shells. In the first test, six independent observers were asked to perform a series of six measurements and observations on the randomly selected sample. The background of the

independent observers was as follows:

1. Two archaeology undergraduates with no previous research experience.
2. One archaeology graduate with a palaeontological background.
3. One archaeology graduate student with no related experience.
4. Two zooarchaeology graduate students engaged in growth line research.

In an attempt to eliminate biases introduced by the researcher, detailed written instructions of the required observations (see Appendix A) were given to each observer and the actual test was supervised by an undergraduate volunteer.

The test results are shown in Table 3. In Table 3(f) the growth ring counts are contrasted with a macroincrement count obtained from thin sections with the aid of a binocular microscope. The margin of error between observers for the metric observations is not great, but it can be readily seen that there is nothing approaching agreement on the number of growth rings or the age of these shells. Table 3(g) shows that removal of the periostracum by means of a potassium hydroxide solution did not improve the reliability of the ring counting results. If a researcher were to construct growth curves based on ring counts which were macroscopically observed, a whole series of significantly different curves could be constructed from the sample depending upon the particular count which was used. It can be readily seen that any estimate of seasonality based on

Table 3. Independent observer data on L. siliquoidea (for methodology see Appendix A).

(a)											
					Length A						
Cat.#	A-006	A-121	A-123	CA-010	CM-003	M-011	M-021	M-031	M-108	M-109	
Obs. 1	87.5	45	48	80	60	83	61	42	79	66.5	mm
2	87	45	40.5	79	59	81	60	42	77	60.5	mm
3	88	44	48	80	60	83	62	42	79	67	mm
4	88	45	48	81	60	82.5	61.5	43	79	67	mm
5	88	45	48.5	81	60	83	42	42.5	79	47	mm
6	88	45	48	81	59	82	62	43	80	52	mm
$\bar{X}$	87.5	44.83	46.83	80.33	59.67	82.42	58.08	42.42	78.83	60.0	
$s/\bar{X}$	0.48	0.91	6.64	1.02	0.87	0.97	13.62	1.16	1.24	14.40	%
s	0.42	0.41	3.11	0.82	0.52	0.80	7.91	0.49	0.98	8.64	

Table 3. continued

(b)		Width B										
Cat.#		A-027	A-035	A-203	A-205	CA-004	CA-007	CA-015	M-019	M-028	M-101	
Obs.	1	39.5	37.5	37	37	38	39	34.5	32	45	44.5	mm
	2	40.5	38	38	47	39	39	33	32	43	41.5	mm
	3	38	36	37	36	37	39	33	32	43	42	mm
	4	38	36	36.5	36	37.5	38	33.5	31.5	43	42	mm
	5	38	36	35	36	37	39	33	31	43	43	mm
	6	40	35	37	38	40	43	35	34	45	43	mm
$\bar{X}$		39	36.42	36.75	38.33	38.08	39.5	33.67	32.08	43.67	42.67	
$s/\bar{X}$		2.92	3.05	2.69	11.27	3.15	4.46	2.61	3.18	2.36	2.53	%
s		1.14	1.11	0.99	4.32	1.20	1.76	0.88	1.02	1.03	1.08	

Table 3. continued

(c)	Thickness C										
Cat.#	A-002	A-017	A-030	A-042	A-119	A-206	A-209	M-022	M-029	M-036	
Obs. 1	14	17	12.5	13.5	5	15	15.5	12.5	8	10	mm
2	11.5	13.5	12	11.5	5.5	13.5	15	11	5.5	5.5	mm
3	13.5	17	12	13.5	5.5	16.5	18	13	8	10	mm
4	13.5	17	12.5	13.5	5	16	18	13	7.5	10	mm
5	14	17	12.5	14	5	16	18.5	13.5	8	10.5	mm
6	11	15	12	13	5	14	15	11	7	8	mm
$\bar{X}$	12.92	16.09	12.25	13.17	5.17	15.17	16.67	12.33	7.33	9.0	
$s/\bar{X}$	10.22	9.32	2.20	6.68	5.03	7.98	9.96	8.76	13.37	21.33	%
s	1.32	1.50	0.27	0.88	0.26	1.21	1.66	1.08	0.98	1.92	

Table 3. continued

(d)	Length D										
Cat.#	A-009	A-013	A-046	A-047	A-106	A-135	A-210	CA-020	CM-001	CM-004	
Obs. 1	23	19	22	18.5	20	17	18	20	18	31	mm
2	24.5	22	21.5	20	19.5	18.5	22	24.5	17.5	33.5	mm
3	23	20	20	19	19	19	20	21	17	28	mm
4	22	20	19	18	20	17	20	21	16	29	mm
5	22	19	17	16	18	15	19	19	15	27	mm
6	24	20	20	18	18	18	20	24	17	35	mm
$\bar{X}$	23.08	20.0	19.92	18.25	19.08	17.42	19.83	21.58	16.75	30.58	
$s/\bar{X}$	4.42	5.50	9.04	7.24	4.82	8.21	6.71	10.19	6.45	10.37	%
s	1.02	1.10	1.80	1.33	0.92	1.43	1.33	2.20	1.08	3.17	

Table 3. continued

(e)	Width E										
Cat.#	A-008	A-122	A-202	A-902	CA-011	CA-012	CM-005	M-023	M-030	M-102	
Obs. 1	45	28	38	47	40	46	45	45	36	46	mm
2	44.5	29	39	48	44.5	48	47.5	45	47.5	45.5	mm
3	43	27	38	45	39	45	43	41	35	45	mm
4	43	26	37.5	46	43.5	45	43.5	41	35	45	mm
5	42	28	38	46	39	45	43	41	35	46	mm
6	45	27	38	46	39	45	43	40	35	45	mm
$\bar{X}$	43.75	27.50	38.08	46.33	40.83	45.67	44.17	42.17	37.25	45.42	
$s/\bar{X}$	2.86	3.82	1.29	2.22	6.12	2.65	4.10	5.43	13.53	1.08	%
s	1.25	1.05	0.49	1.03	2.50	1.21	1.81	2.29	5.04	0.49	

Table 3. continued

(f)		Annuli count c/w periostracum										
Cat.#		A-045	A-207	CA-003	CA-016	CM-006	M-004	M-032	M-103	M-104	M-106	
Obs.	1	53	65	44	31	59	44	57	45	56	65	rings
	2	23	21	16	24	25	13	23	16	12	15	rings
	3	13	12	9	11	12	4	5	8	9	9	rings
	4	8	8	9	10	11	3	7	6	9	9	rings
	5	14	12	10	13	14	10	14	8	10	14	rings
	6	10	11	10	10	11	5	6	7	6	21	rings
Micro Count		30	24	13	17	29	7	17	8	20	18	rings
Observers only	$\bar{X}$	20.17	21.50	16.33	16.50	22.00	13.17	18.67	15.00	17.00	22.17	
	$s/\bar{X}$	83.74	101.16	84.57	53.76	85.86	118.38	106.96	100.87	112.97	96.75	%
	s	16.89	21.75	13.81	8.87	18.89	15.59	19.97	15.13	19.20	21.45	
	Range	45	57	35	21	48	31	52	39	50	56	

Table 3. continued

(g)	Annuli count with periostracum removed										
Cat.#	A-045	A-207	CA-003	CA-016	CM-006	M-004	M-032	M-103	M-104	M-106	
Obs. 1	13	7	8	11	10	8	5	9	12	12	rings
2	19	37	14	29	17	13	26	32	35	22	rings
3	14	12	7	16	12	4	13	7	12	14	rings
4	11	11	10	10	11	6	7	7	9	9	rings
5	25	23	26	23	27	17	21	20	20	25	rings
6	13	10	7	12	11	5	11	6	10	9	rings
Micro Count	30	24	13	17	29	7	17	8	20	18	rings
$\bar{X}$	15.83	16.67	12.0	16.83	14.67	8.83	13.83	13.5	16.33	15.17	
$s/\bar{X}$	33.04	68.15	61.25	45.34	44.51	58.44	59.00	77.41	66.81	44.76	%
s	5.23	11.36	7.35	7.63	6.53	5.16	8.16	10.45	9.93	6.79	

any of these curves would be no more reliable than an offhand guess.

In the second test a graduate student engaged in growth line research was asked to perform the same observations on five different occasions. Once again the metric observations were within acceptable limits, but the variation in the counts of the growth rings was unacceptably high (Table 4).

It is evident that establishing criteria for the definition of growth rings in L. siliquoidea is not a simple matter. It is also apparent that the application of criteria by a single observer using macroscopic techniques is not consistent or reliable.

A third major problem associated with interpreting seasonality based on comparison of increment size to a growth curve is that the observed width of a macroincrement is an extremely poor indicator of the season in which the mussel died or of the length of time the animal had been depositing a macroincrement. In figure 3 the thickness, in microns of the most recently deposited macroincrement in each of the shells collected at station three, is plotted as a function of the date of collection. The data points are randomly scattered, and the range of overlap is almost complete. When the sample averages are calculated for each collection day, it is found that the first and the last samples collected fall next to each other in the center of the range of sample averages. It is apparent that with freshwater mussels there is no good evidence to support the notion that seasonality can be inferred from the examination of

Table 4. Individual observer data on L. siliquoidea.

(a)	Length A										
Cat.#	A-006	A-121	A-123	CA-010	CM-003	M-011	M-021	M-031	M-108	M-109	
Aug. 29	88	45	48	81	60	82.5	61.5	43	79	67	mm
Nov. 19	87	44.5	48	80.5	60	82	61.5	42	79	67	mm
20	88.5	44.5	47.5	80.5	60	82.5	61.5	42	79	66.5	mm
26	88	45	48	80.5	60	82.5	62	42	79.5	67	mm
27	88	44.5	48	81	60	83	62	42	79.5	67	mm
$\bar{X}$	87.9	44.7	47.9	80.7	60	82.5	61.7	42.2	79.2	66.9	
$s/\bar{X}$	0.63	0.60	0.46	0.33	0.0	0.42	0.44	1.07	0.34	0.33	%
s	0.55	0.27	0.22	0.27	0.0	0.35	0.27	0.45	0.27	0.22	

Table 4. continued

(b)	Width B										
Cat.#	A-027	A-035	A-203	A-205	CA-004	CA-007	CA-015	M-019	M-028	M-101	
Aug. 29	38	36	36.5	36	37.5	38	33.5	31.5	43	42	mm
Nov. 19	38	36.5	34	36	36	36.5	33.5	30.5	43.5	42	mm
20	38.5	36.5	35	35.5	36	36.5	34	31	42	42	mm
26	38.5	36	35	35	36	36.5	33.5	31	42.5	42.5	mm
27	38.5	36	34.5	36	36.5	37.5	33.5	32	43	42	mm
$\bar{X}$	38.3	36.2	35	35.7	36.4	37	33.6	31.2	42.8	42.1	
$s/\bar{X}$	0.70	0.75	2.69	1.26	1.79	1.92	0.65	1.83	1.33	0.52	
s	0.27	0.27	0.94	0.45	0.65	0.71	0.22	0.57	0.57	0.22	

Table 4. continued

(c)	Thickness C										
Cat.#	A-002	A-017	A-030	A-042	A-119	A-206	A-209	M-022	M-029	M-036	
Aug. 29	13.5	17	12.5	13.5	5	16	18	13	7.5	10	mm
Nov. 19	14	16.5	12.5	13.5	5	16.5	18	13	7.5	10	mm
20	13.5	16.5	12	13.5	5	16.5	18	13	7.5	10	mm
26	13.5	16.5	12	13.5	5	16.5	18	12.5	7.5	10	mm
27	13.5	16.5	12	13.5	5	16	17.5	12.5	7.5	10	mm
$\bar{X}$	13.6	16.6	12.2	13.5	5	16.3	17.9	12.8	7.5	10	
$s/\bar{X}$	1.62	1.33	2.21	0.0	0.0	1.66	1.23	2.11	0.0	0.0	%
s	0.22	0.22	0.27	0.0	0.0	0.27	0.22	0.27	0.0	0.0	

Table 4. continued

(d)					Length D						
Cat.#	A-009	A-013	A-046	A-047	A-106	A-135	A-210	CA-020	CM-001	CM-004	
Aug. 29	22	20	19	18	20	17	20	21	16	29	mm
Nov. 19	20	19	19	18.5	19	18	20	21	16	29	mm
20	21	19	18.5	19	18	17.5	20	21	16	27	mm
26	22	19	20	18.5	19	18	19	20.5	16	28	mm
27	22	20	19	19	19	18	20	20.5	16	29	mm
$\bar{X}$	21.4	19.4	19.1	18.6	19	17.7	19.8	20.8	16	28.4	mm
$s/\bar{X}$	4.16	1.39	2.88	2.26	3.74	2.54	1.11	1.30	0.0	3.13	%
s	0.89	0.27	0.55	0.42	0.71	0.45	0.22	0.27	0.0	0.89	

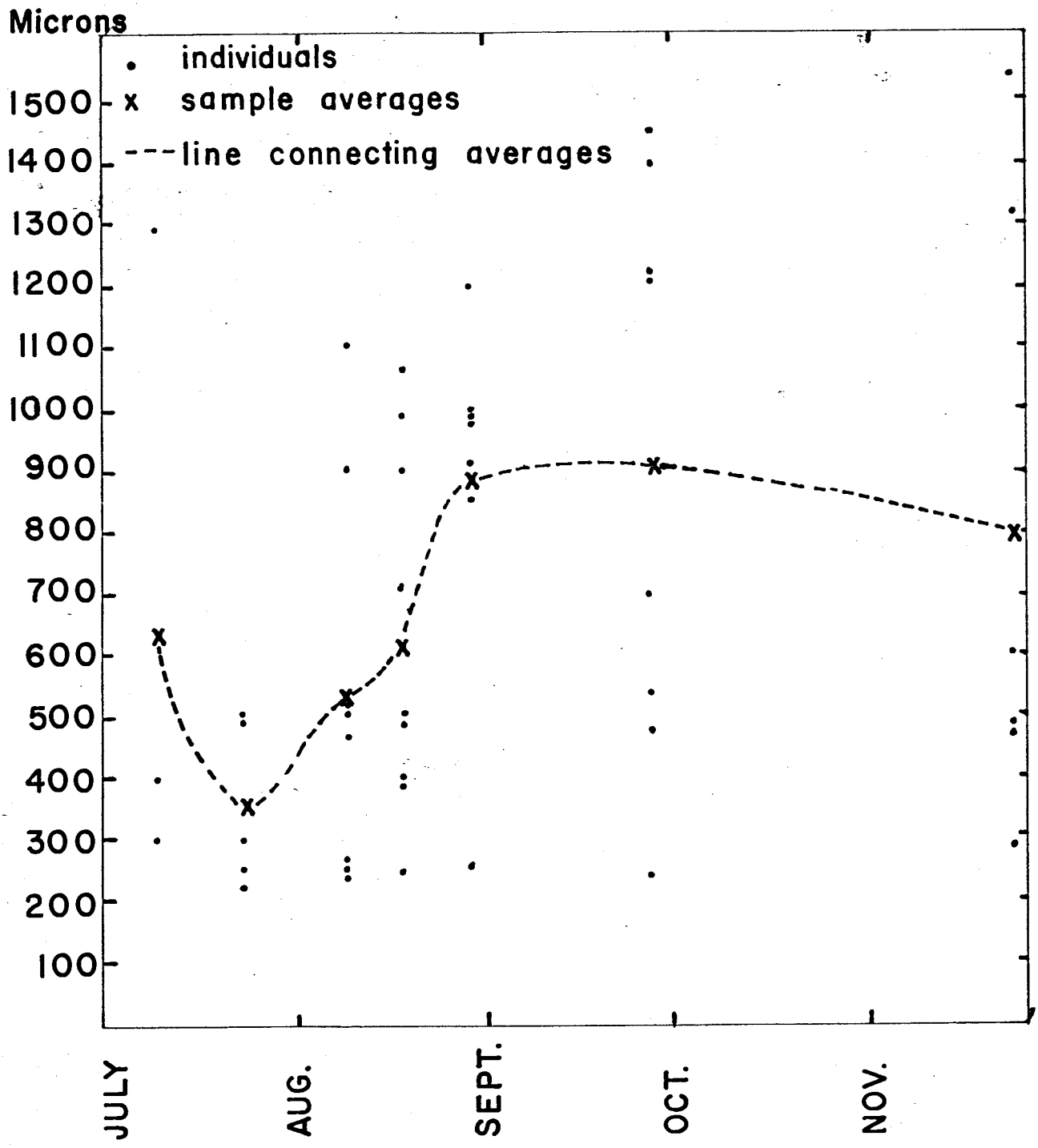
Table 4. continued

(e)											
					Width E						
Cat.#	A-008	A-122	A-202	A-902	CA-011	CA-012	CM-005	M-023	M-030	M-102	
Aug. 29	43	26	37.5	46	43.5	45	43.5	41	35	44.5	mm
Nov. 19	43	25.5	37.5	46	43.5	45	43	41	35	44.5	mm
20	43	27	37.5	45.5	38	45	43	41	35.5	45	mm
26	44	27	38	45.5	38.5	44.5	43	41.5	35.5	45.5	mm
27	43.5	27.5	38	45.5	38.5	45	43	41	35.5	45	mm
$\bar{X}$	43.3	26.6	37.7	45.7	40.4	44.9	43.1	41.1	35.3	44.9	
$s/\bar{X}$	1.04	3.08	0.72	0.59	7.03	0.49	0.51	0.54	0.76	0.94	%
s	0.45	0.82	0.27	0.27	2.84	0.22	0.22	0.22	0.27	0.42	

Table 4. continued

(f)	Annuli count										
Cat.#	A-045	A-207	CA-003	CA-016	CM-006	M-004	M032	M-103	M-104	M-106	
Aug. 29	8	8	9	10	11	5	7	6	9	9	rings
Nov. 19	11	11	10	10	11	5	9	7	9	9	rings
20	11	10	8	12	11	4	11	7	10	10	rings
26	13	13	8	12	12	4	10	7	10	9	rings
27	8	11	7	12	11	4	7	6	10	8	rings
Micro Count	30	24	13	17	29	7	17	8	20	18	rings
$\bar{X}$	10.2	10.6	8.4	11.2	11.2	4.4	8.8	6.6	9.6	9	rings
$s/\bar{X}$	21.27	17.17	13.57	9.82	4.02	12.50	20.34	8.33	5.73	7.89	%
s	2.17	1.82	1.14	1.10	0.45	0.55	1.79	0.55	0.55	0.71	
Range	5	5	3	2	1	1	4	1	1	2	rings

Figure 3. Macroincrement Thickness in Microns Expressed as a Function of the Date of Collection



their shells by macroscopic techniques. This view is supported by the data in Table 5 which shows that there is no demonstrable relationship between macroincrement thickness and the included number of microincrements, or the date of collection.

It must be concluded that the investigation of seasonality through growth ring analysis in L. siliquoidea cannot be pursued by macroscopic visual examination. The results of the observer tests demonstrate that there is a wide variation in the criteria which independent observers use to interpret growth rings, and between the actual microscopically observed number of macroincrements. The single observer test also shows an unacceptable amount of variation in the growth ring counts. In addition, it is evident that the relative increment thickness is an unreliable indication of seasonality. The meager literature dealing with freshwater mussels offers no reliable criteria by means of which it is possible to establish the origin of each growth ring appearing on the surface of the valves. Any attempt to derive seasonality from such tenuous and subjective data is worse than useless. It is actually misleading and can lend a false sense of authority to an incorrect interpretation. Clarke (1973:68) in his study of the mussels of the Canadian Interior Basin notes:

> "An attempt was also made to count growth annuli on the specimens collected. It was impossible to decide whether some of the lines represented annual events or not, however, and the value of the results obtained (after examining hundreds of specimens) was therefore

Table 5. Lampsilis siliquoidea collected at station 3 showing variation in the first three macroincrements of the thickness (in microns) and the number of included microincrements in relation to the date of collection.

Catalogue Number	Date of Collection	Macroincrement Number	Thickness in microns	Microincrement Count
A-001	July 8/79	1	1290	17
		2	750	85
		3	560	79
A-008	July 23/79	1	490	12
		2	410	36
		3	420	47
A-016	Aug. 8/79	1	250	35
		2	130	47
		3	400	58
A-031	Aug. 29/79	1	850	48
		2	1750	76
		3	1250	122
A-049	Sept. 28/79	1	240	46
		2	1360	117
		3	490	115
A-069	Nov. 26/79	1	600	84
		2	920	98
		3	550	59
A-070	Nov. 26/79	1	1310	76
		2	790	127
		3	380	63

considered minimal."

The findings of this investigation are in full support of the pessimistic conclusions of Clarke (1973) with regard to external growth rings on freshwater mussels.

If a reasonable estimate of seasonality can be derived from the shells of freshwater mussels, it must be obtained from a study of the microstructural elements of the shell. There is a substantial palaeontological literature indicating that with several marine species of bivalves such studies are feasible (Barker 1964,1970; Berry and Barker 1975; Clark 1968, 1974a,1974b,1975; Crenshaw and Neff 1969; Cunliffe and Kennish 1974; Dolman 1975; Evans 1972,1975; Farrow 1971; Hall 1975; Hall et al.1974; House and Farrow 1968; Kobayashi 1969; Koike 1973,1975,1979; Kennedy et al. 1969; Lutz and Rhoads 1977; Millar 1968; Neville 1967; Pannella and MacClintock 1968a, 1968b; Pannella 1975; Rhoads and Pannella 1970; Richardson et al. 1979; Runcorn 1968; Thompson 1975; Whyte 1975). This thesis will attempt to demonstrate that any inferences of seasonality made from the shells of freshwater mussels must be based on an interpretation of the microstructure of the shells rather than upon macroscopic observations.

The earlier belief that there was a linear relationship between the biological response of mussels, expressed by the patterned deposition of shell increments, and the passage of the seasons, or to synodical periodicity, has been shown to be untrue. The wide range of variables affecting marine and freshwater mussels as individuals and as assemblages have been

shown to alter regular patterns of shell deposition. It has been shown that it is extremely difficult to identify and differentiate between growth lines initiated by different causal agencies. The observer tests, conducted as a part of this research, confirm that a serious problem exists in the identification of growth rings in freshwater mussels. Finally, when the thickness of the most recently deposited macroincrement was plotted as a function of the date of collection, there was no indication of a regular seasonal progression of accumulated shell deposition.

Chapter 3: LABORATORY TECHNIQUES

Most archaeological shell recovered from prehistoric sites has the carbonate surface exposed because of the disintegration of the organic periostracum. Growth rings are evident on most of these shells. To determine whether the growth rings might be more accurately counted by macroscopic methods if they were not covered by the periostracum, this layer was removed from the sample which had been used for the annuli counts in the observer error tests.

To remove the periostracum, the left valves were soaked in warm water overnight and then placed in a potassium hydroxide solution for 48 hours. The valves were then rinsed under running tap water and scrubbed with a commercial woven plastic pot scouring pad. This technique effectively removed the periostracal layer without damaging the underlying prismatic layer. It was found that if the valves were left in the potassium hydroxide solution for periods much in excess of 48 hours the prismatic layer tended to separate and peel away from the nacreous structure of the underlying mesostracum. The valves which were treated in this manner were similar in appearance to specimens which have been recovered archaeologically.

In order to interpret the microstructure of the mussel shells in this sample, two methods of light microscope

investigation were evaluated. In addition, several specimens were mounted and studied using the ETEC Autoscan scanning electron microscope.

The light microscope techniques required that radial sections be cut through the umbo to the ventral edge of the shell. This was done with a precision Isomet saw. In all cases the right valve was sectioned. Prior to cutting, the valves were soaked for several hours in warm water. This procedure softened the periostracum and minimized chipping or peeling of material during the cutting process. Water soaking is not an adequate preparation for archaeological shell when the periostracum has disintegrated. In such cases it is necessary to coat the shell with epoxy or embed it in some medium such as bakelite (Dolman 1975), resin (Koike 1973) or Plaster of Paris (Ray 1976) to prevent the shell from disintegrating due to blade torque of the saw. The prismatic layer is especially susceptible to crumbling, and the nacreous layers to foliation, during cutting.

The first of the light microscope methods considered was the preparation of acetate peels. The cut section was first smoothed on a glass plate using 925 grit with water as a lubricant. The cut surface was etched with 10 percent hydrochloric acid (HCl) for 20 seconds following the smoothing procedure, and then it was flushed under running water. The etched shell was allowed to air-dry. A strip of six mil acetate was softened with acetone and the prepared shell

section impressed firmly upon it. Additional acetone was swabbed along the edge of the section and the acetate strip impressed firmly against the cut, etched surface. The peel was allowed to dry for 15-20 minutes then carefully stripped from the shell. The peel obtained in this manner can be examined under a microscope directly with reflected light, or mounted on a glass slide prior to study. The technique gives good resolution of large scale structures such as macroincrements and the myostracum. However, the negative impressions of microincrements were spotty and extremely difficult to obtain in the freshwater species under study. In addition, there was a tendency for crystals in the prismatic layer to adhere to the peel and be plucked out of the etched section. This second defect rendered the technique almost useless for archaeological shell. As a consequence of these problems, it was decided to mount and stain thin sections to study the growth lines in the infrastructure of the shells in the sample.

The initial stage of cutting and smoothing the radial shell section was the same as that used for acetate peel preparation. However, instead of etching, the smoothed cut was mounted on petrographic slides with Hillquist A/B epoxy. The mounted shells were cured for one hour at 130 degrees Celsius on a slide warmer. Following the curing process, the petrographic slides were mounted on a vacuum chuck on the dolly arm of the Isomet saw. Using the micrometer adjustment, the glass slide was brought into contact with the diamond

blade and then backed off approximately 80 microns (.003 of an inch). The shell was then cut away from the slide leaving an attached section approximately 70-100 microns thick. The mounted section was smoothed on a glass plate using 925 grit with water as a lubricant. The section was then stained with Toluidine Blue O stain.

The procedure used to stain the thin sections was as follows:

1. Etch the sections for 20 seconds in a 10 percent hydrochloric acid solution.
2. Rinse the section under running water.
3. Place the section in formol saline solution for at least eight hours (Appendix C).
4. Rinse the section in distilled water.
5. Place the section in a Toluidine Blue O solution for four hours (Appendix C).
6. Rinse the section in distilled water.
7. Allow the section to air dry for about one hour.
8. Mount glass cover slips with FLO-TEXX mounting medium (Appendix C).

The etching procedure was used to expose a more concentrated area of conchiolin to the formol fixative for subsequent staining. It was found that Toluidine Blue O emphasized the large scale structures within the shell infrastructure more clearly than did an Alizarin Red S solution. Both stains were of value in enhancing the microstructural

lines. It was found that the Hillquist C/D mounting epoxy extracted the stain from the sections and dispersed it throughout the mounting medium. For this reason, the FLO-TEXX product was used to mount the cover slips, as it did not affect the stain. In a few cases, the slides were left for too long a period in the Toluidine Blue O solution. This resulted in excessive staining which interfered with counting the microincrements through the light microscope. It was found that if the slide was etched for two or three seconds with a 10 percent hydrochloric acid solution and immediately rinsed in distilled water, prior to mounting the cover glasses, the excess staining could be removed.

To determine whether additional information could be obtained by means of the Scanning Electron Microscope (SEM), a number of specimens were prepared to do a comparative study. The preparation of the study specimens was less complicated than either thin sections or acetate peels. However, the SEM did not provide any significant additional information. Radial sections were cut from the ventral edge of the valves with the Isomet saw and mounted on SEM studs with carbon paste. They were etched for 10 seconds with 10 percent hydrochloric acid and then rinsed under running water and air-dried. The specimens were vacuum coated with gold and examined with the SEM.

The SEM shows growth lines on selected prismatic crystals satisfactorily at 10^3. However, the field of view is so small

that, given the discontinuous nature of many of the microincrements, it would be extremely difficult to do a growth line count unless an entire section of shell were photographed and a large composite photograph of the shell constructed. While the cost in time and money for an individual shell would not be excessive, the cost for an assemblage would be very high. The use of the SEM did not furnish any additional information on the microincrements in the prismatic layer and none were visible in the nacreous structure.

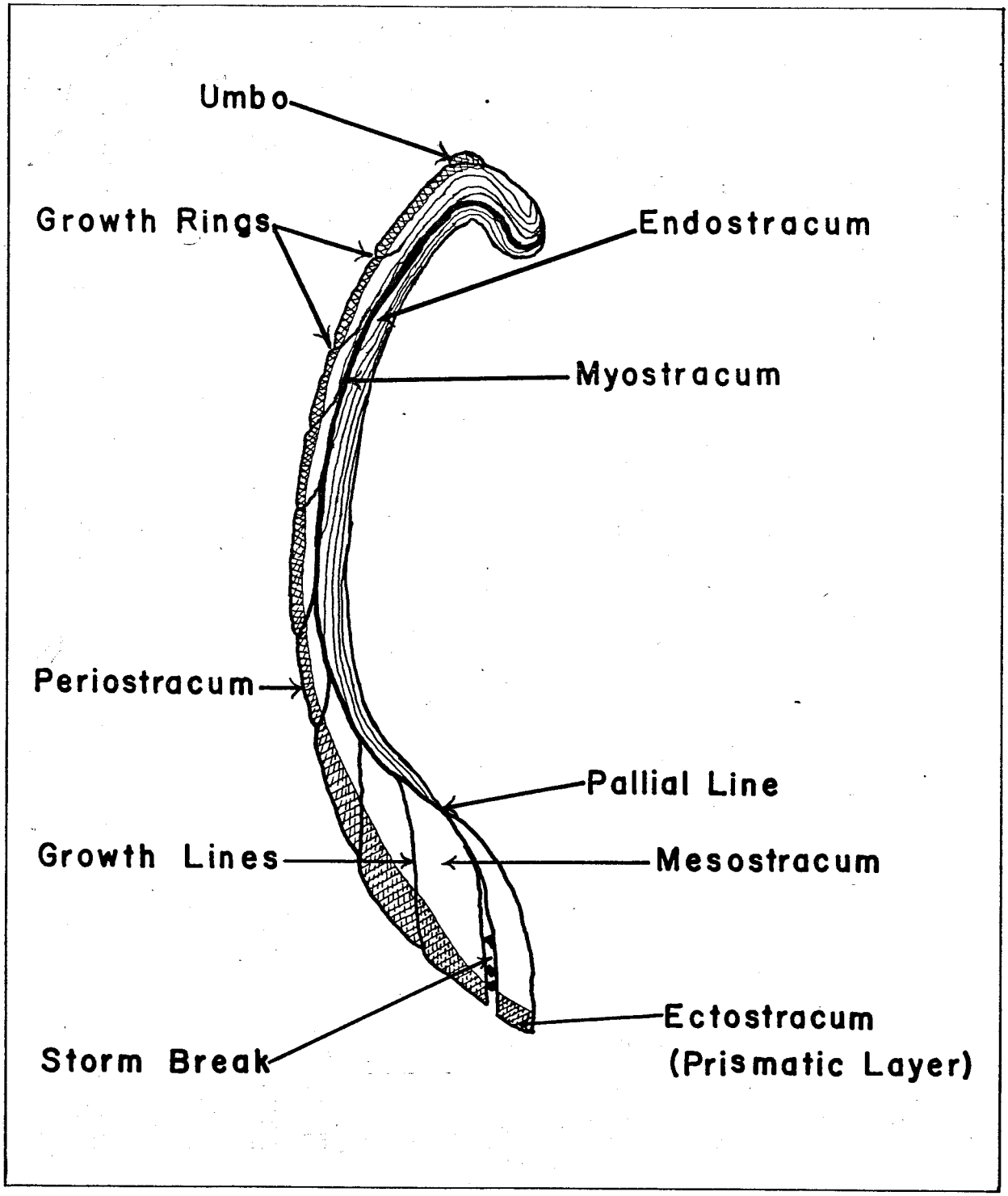
The technique may be an alternative to thin sections in the study of fragile archaeological specimens, but it would be a slow and expensive way of processing large samples if composite photographs were necessary. The use of stained thin sections and a light microscope proved to be the most useful technique to study the microstructure of the mussels in this sample.

Chapter 4: MICROSCOPIC EXAMINATION OF INFRASTRUCTURE OF LAMPSILIS SILIQUOIDEA

The exoskeleton of a freshwater unionid is composed of three distinct carbonate layers in addition to the exterior periostracum which is essentially a deposit of the albuminoid substance conchiolin (Figure 4). Wilbur (1960:18) describes shell infrastructure as follows: "Each main shell layer is a multilayered structure of crystalline material, the lamellae of which are separated by conchiolin." The boundaries or growth lines which separate the lamellae represent interruptions in deposition of the shell material. These discontinuities occur in response to environmental and physiological events experienced by the individual mussels. A demonstrated relationship between the cumulative frequency of growth line deposition and the passage of measureable units of time would form a basis for the estimation of seasonality.

It has been demonstrated that marine bivalves reflect the effects of environmental variables through an immediate response in their metabolic processes (Barker 1964,1970; Clark 1975; Cunliffe and Kennish 1974; Evans 1972,1975; Hall 1975; Hall et al. 1974; Koike 1973; Kennish and Olsson 1975; Lowenstam 1954; Pannella and MacClintock 1968a,1968b; Rhoads and Pannella 1970; Rosenberg and Jones 1975; Thompson 1975; Whyte 1975). Moderate temperature shifts often alter the

FIGURE 4. Radial cross section of a freshwater mussel shell



crystal polymorph which the animal has been depositing, but if they are subjected to more drastic, unfavorable environmental conditions, they will withdraw into their shells, and metabolism either ceases or shifts from aerobic to anaerobic processes (Lutz and Rhoads 1977). In the first case, shell deposition ceases, and in the second, there may be an actual resorption of carbonate material from the shell surface to buffer acids, especially succinic acid, produced by the anaerobic metabolic process. The processes which have been demonstrated to disrupt the deposition of shell in marine bivalves and initiate recognizable incremental structures are:

(a) solar year . . . . . macroincrements
(b) storms . . . . . macroincrements
(c) spawning . . . . . macroincrements
(d) predator attacks . . . . . macroincrements
(e) thermal shocks . . . . . macroincrements
(f) freeze shocks . . . . . macroincrements
(g) tidal cycle (fortnight) . . . microincrement cluster
(h) diurnal (circadian) . . . . . microincrements
(i) daily tide rhythm . . . . . microincrements

Deposition of shell takes place when the valves are open and water is passing through the siphon and over the gills. The shell is composed of calcium carbonate in the form of aragonite or calcite crystals deposited on an organic matrix. In the Unionidae, of which L. siliquoidea is a member, the shells are composed of an outer prismatic layer and inner

nacreous layers (Kobayashi 1969:669). The outermost layer, the palliostracum, is composed of two crystalline structures having a distinctly different orientation of the Z or long axis. The prismatic outer sublayer, the ectostracum, has the long axis roughly perpendicular to the growth lines, and the nacreous sublayer, the mesostracum, has the long axis of the crystals roughly parallel to the growth lines. The palliostracum is secreted by the ventral folds of the mantle. The middle layer, the myostracum, is a deposit of the pallial line. The pallial line is a boundary between the dorsal and ventral mantle which is loosely attached to the valves. In cross-section, the myostracum appears to crosscut the major growth lines. The inner layer, the endostracum, is secreted by the dorsal portion of the mantle. In consequence of this layered structure, the major growth lines pass through several layers deposited by distinctly different portions of the mantle. Any interpretation of growth lines must take into account the possibility of one portion of the mantle depositing shell independently of the other portions. Examination of the present sample indicated that this was the case for many of the valves (Plate 1). Often, the number of macroincrements visible in the umbo did not coincide with the number of macroincrements which were observed in the palliostracum.

In examining the thin sections microscopically, a macroincrement was defined as a segment of shell in the palliostracum bounded by well defined and stained growth lines which extended from the periostracum through the ectostracum

Plate 1: Myostracum Crosscutting Growth Lines at the Boundary of the Mesostracum and the Endostracum

and mesostracum to the inner surface of the shell, or to the myostracum. Well defined growth lines which terminated before entering the mesostracum or which faded out before reaching either the interior surface of the shell or the myostracum were treated as minor disturbance lines rather than annuli. The segments which they bounded were combined to form single macroincrements when the microincrement counts were made. All of the counts listed in Appendix B were treated as microincrement totals found within macroincrements, and were bounded by growth lines believed to represent winter hiatuses. It is recognized that, in many cases, the macroincrements may represent some period less than a season of growth, but no internal criteria could be identified for making any reliable, objective separations between the macroincrements resulting from different environmental or physiological conditions.

Two mussels, A-052 and A-054, which contained abnormally low microincrement counts, in terms of the assemblage of which they are a part, show indications of shell trauma which may have been induced by disease or parasites (Plate 2). These specimens are excluded from the averages in Figure 4, as are A-12 and A-15 which showed recent shell damage.

In all of these cases, the damage to the shell was obvious. The identification of these traumas in no way depended upon prior knowledge of the shell microstructure or upon the examination of soft tissue. It is reasonable to assume that valves which showed evidence of damage or

Plate 2: Mussel Valve Showing Interior Lesion

A-054

deformity would reflect in their infrastructure the environmental or biological traumas which induced the observed abnormalities. All damaged or deformed shells should be culled from assemblages to be used for seasonality studies.

One of the demonstrated initiators of major growth lines in marine species is spawning (Kennish and Olsson 1975). There is no evidence of a pattern of major growth lines being formed in this sample during summer, on either a population level, or along sexual lines in L. siliquoidea, or any of the other species examined. It is known that the Unionidae release their glochidia from late June until early August (Clarke 1973; Matteson 1948). Glochidia were present in females in this sample collected after mid-August. While individual physiology is important, beyond a reasonable doubt, to patterns of deposition in any given mussel, there is no obvious biological rhythm imposed on the growth line patterns found in the infrastructure at the population level in this sample.

The microincrement counts were made using a Nikon binocular microscope and transmitted light at 400X magnification. The conchiolin rich layers showed up as bright translucent bands with the aragonite bands appearing darker. The stain appeared to enhance the boundaries between the layers.

The microincrements are visible only in the prismatic layer of the species included in this sample. The individual, plate-like aragonite crystals in the mesostracum can be

readily distinguished in cross section, but there are no continuous structures which could be identified as microincrements. The conchiolin bands, which form the boundaries of the microincrements pass through the individual prismatic crystals at approximately right angles to their long axes. The prismatic crystals are, in general, continuous from their point of origin at the periostracum until they terminate at their juncture with the mesostracum (Figure 4).

A major problem in the definition and identification of microincrements was the frequent lack of continuity of growth lines through a continuous series of prismatic crystals. In many cases, a growth line which had passed through several crystals would abruptly cease at a crystal boundary and then reappear several crystals further on (Plate 3). The reason for this is unknown. A micrometer controlled microscope stage is invaluable for controlling slide movement while tracing these discontinuous lines.

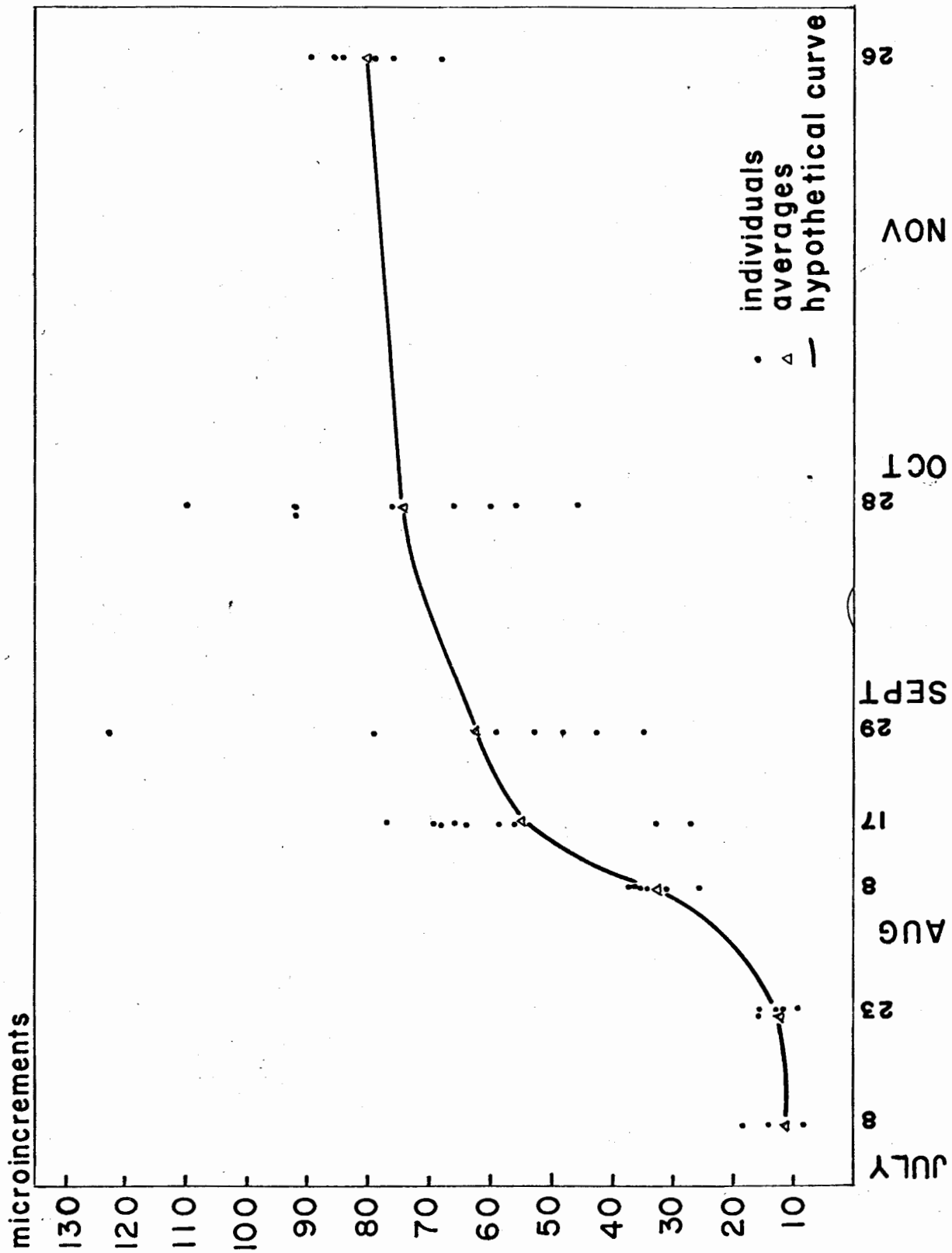
Criteria for delineation of microincrements were extremely difficult to define, especially at the margins of the macroincrements. In the slow growth regions, at the outer margins of many of the macroincrements, which are assumed to occur in spring and fall, the boundaries of the microincrements are extremely close together and grade slowly into what appear to be subdaily increments during the season of more rapid growth, assumed to be summer.

Plate 3: Discontinuous Microincrements

With practice, it became increasingly apparent that light transmitting properties of the conchiolin accounted for light and dark banding and that there was a recognizable difference between the boundaries of increments and the gradual changes which occurred within increments. These differences were most apparent during the periods of rapid and slow growth. In the transitional regions of the macroincrements, the differences were less well marked. There is a measure of subjectivity in the counts of the microincrements, but the overall trend towards an increasing average number of microincrements in the final ventral macroincrement, as the season progresses, is apparent in Figure 5.

The criteria used to differentiate between growth checks in marine bivalves do not appear to be reliable in evaluating the structures of freshwater mussels. The ameliorating effect of the ocean on short term temperature fluctuations is not as great in rivers and freshwater lakes where the volume of water is much less. A sudden drop in air temperature or a sudden rise in air temperature is quickly expressed in a corresponding drop or rise in freshwater temperature. At a latitude of 50 degrees North, in a given year, there may not be a period of slow growth preceding the winter hiatus. This would occur if there was a sudden and unremitted drop in air temperatures in the early fall while growth was still rapid. In addition, confusion could result from the interpretation of hiatuses containing grit particles as storm breaks. The dislodgement of mussels by the spring runoff, prior to the resumption of

FIGURE 5. Microincrements expressed as a function of the date of collection



growth following the winter hiatus, might cause silt and gravel to become lodged beneath the mantle and incorporated into the shell as a feature of a major growth line. Finally, to further obfuscate the record of shell deposition, in the absence of tidal entrainment of the circadian rhythm, the mussels in this sample appear to have developed a highly individualistic response to the variables of their environment. This variability is shown in Figure 5 by the range of microincrement counts in each of the samples gathered from a single mussel bed at Station 3 in the Assiniboine River. For example, in the sample collected August 29, the microincrement count ranges from 35 to 123 in the last macroincrement which was being deposited.

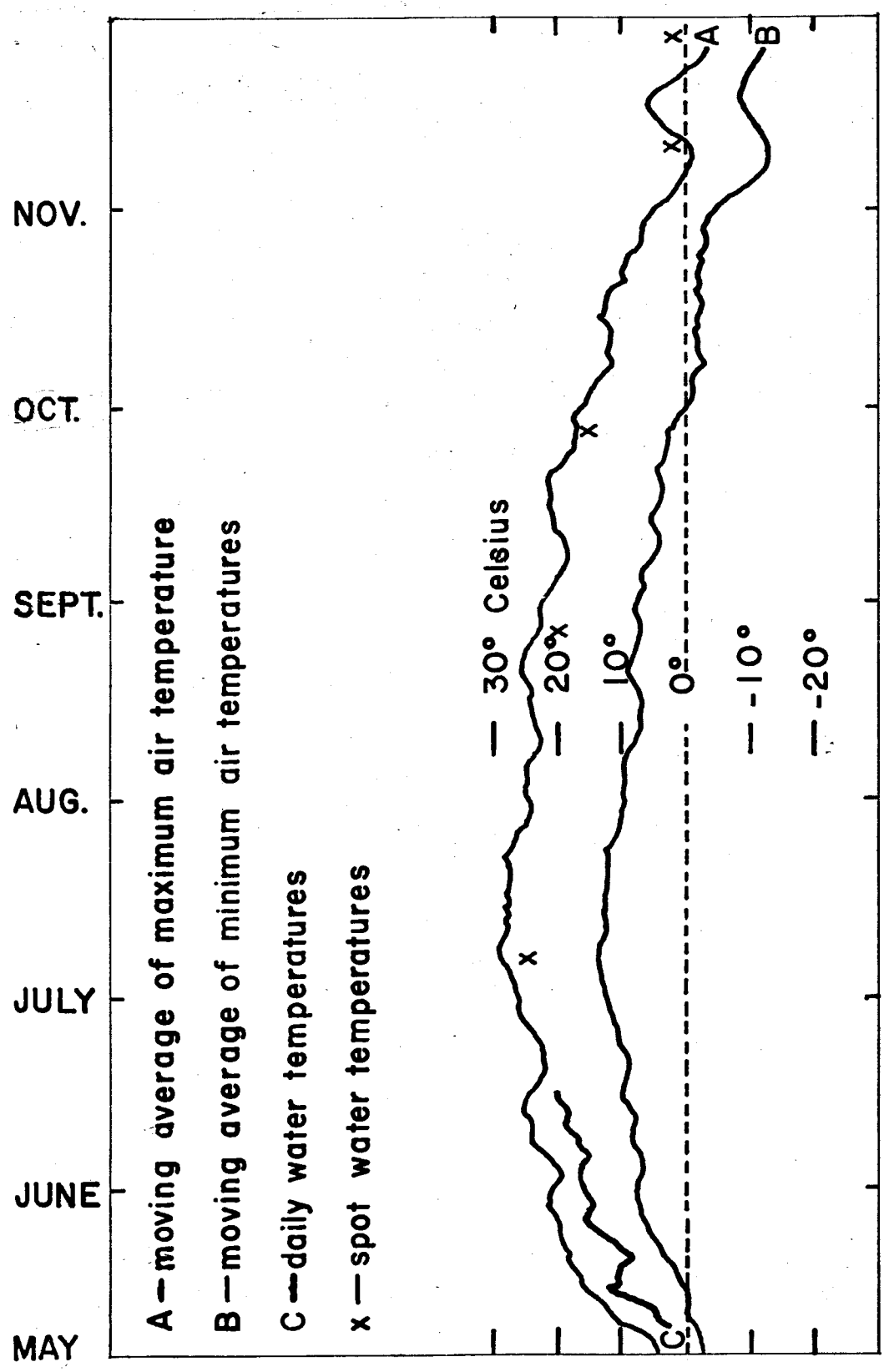
The sample, from which the data for Figure 5 were derived, was drawn from a single mussel bed where the substrate and flow of water were constant. The mussels were collected from a depth of between 20 and 50 cm, and in all respects these mussels appeared to be individually subjected to the same environmental conditions. Yet there is a great variation in the number of microincrements which they recorded. The basis for the response is probably a circadian diurnal rhythm. However, there may be a highly individualistic response to variations of temperature. Several specimens (Plate 4) show small scale hiatuses in the ectostracum which may reflect either heat or cold shocks. There is no way of knowing how great a period of time is represented by these minor checks,

Plate 4: Major and Minor Growth Lines in the Prismatic Layer of a Mussel Valve

or of knowing for certain when they occurred. Successful live staining and confinement experiments would give this information. In view of the difficulty experienced in establishing criteria, by means of which the observer could identify and differentiate between growth checks initiated by different causal agencies, together with the wide variation in the number of microincrements contained in a macroincrement, it is necessary to approach the question of seasonality on the basis of sample averages. In Figure 5, the sample from station three clearly indicates a trend to a greater average number of increments as the growth season progresses. The tentative end of season average would appear to be greater than 60 increments, ranging upwards to 80 increments. This estimate is based on the averages calculated from the microincrement counts in the last three macroincrements of the mussels in the sample collected from station three (Appendix B).

It is evident from an examination of Figure 5 that the period of maximum average microincrement deposition in this sample took place after July 23 and before August 17 following a cumulative rise in mean temperature (Figure 6) and an attendant increase in the population of micro-organisms which these filter feeders consume. It is probable that optimum conditions of temperature and food supply were reached in July, and that following the release of the last of their glochidia, the mussels quickly achieved a maximum metabolic rate of recovery and regeneration. This environmental and metabolic optimum may have been abruptly

Figure 6. Moving Averages of Air Temperatures (taken 11 at a time) between May 6 and November 25



cut short by a severe cold shock on August 13 (Figure 2). The observed variability in the sample microincrement counts may reflect individual metabolic variability influenced by genes, stage of gametogenesis, pathogens, parasites or other unknown physiological factors. However, in spite of this observed variability of response to apparent constant astronomical and environmental stimuli, there is a steady increase in the average microincrement count in successive samples. This observation suggests that the microscopic examination of the infrastructure of the shells of freshwater mussels may provide a reliable seasonal indicator in archaeological sites where a suitable sample can be recovered and analysed for microincrement counts.

Studies of marine bivalves (Andrews 1972; Ansell 1968; Barker 1970; Hall 1975; Hall et al. 1974) indicate that changes in latitude and climatic variables will severely limit the area over which seasonality may be generalized from a set of averages calculated from a standard sample. Each study area and geographic region will require a modern sample from which to estimate a set of seasonal averages. It will also be necessary to determine whether the palaeo-environment of the period under study was essentially similar to modern conditions. It is abundantly clear that a great deal more work is required in this field.

The depositional patterns in bivalve shells reflect the animal's response to changes in their environment or

physiology. Individual mussels appear to demonstrate a highly individualistic response to the same environmental conditions and do not necessarily reflect this response in all layers of their shells. Microincrements are visible only in the ectostracum. The average microincrement count of sequentially gathered samples of mussels shows a steady increase in the microincrement averages with the passage of time during the growth season. This sample average count may form the basis for seasonality estimates in a discrete archaeological assemblage.

Chapter 5: A TENTATIVE EVALUATION OF THE POTENTIAL OF THE OTHER SPECIES IN THE SAMPLE

With the possible exception of Lampsilis ovata and Ligumia recta, the samples of the species other than L. siliquoidea are too small to make a confident evaluation of their growth line study potential. All species in the sample, with the exception of Fusconaia flava, were examined in thin section. In general, all species showed microincrements with varying degrees of definition. All final macroincrements contained counts of microincrements which fell within the range of the L. siliquoidea sample. Samples ofthe other species, with the exception of L. ovata which showed poor definition of microincrements, were too small to evaluate whether or not their patterns of deposition, or frequency of microincrement deposition, were significantly different from those of L. siliquoidea. An extremely tentative statement of their potential, relative to L. siliquoidea, follows:

Amblema plicata - This species appears to have the same structural elements as L. siliquoidea with microincrements which are at least as well defined. A minor hiatus was noted in two specimens collected at station four which, based on the evidence from a backcount from the shell/mantle interface, could result from the August 13 freeze shock (Figure 2).

Anodonta grandis - This species lays down microincrements in a pattern similar to L. siliquoidea but either over an extended growing season or with agreater frequency. The definition of the microincrements appears to be slightly better.

Fusconaia flava - No evaluation of this species was made, due to difficulties in the preparation of the thin-sections. These problems were of a technical nature unrelated to the structure of the shell specifically.

Lampsilis ovata - This closely related species was similar to L. siliquoidea in all respects of shell deposition except that the microincrements were consistently less well defined.

Lasmigona complanata - This species appears to have the most clearly defined microincrements of any of the species examined. On the basis of an extremely small sample, it may also be stated that this species appears to display the most consistent depositional record of microincrements.

Ligumia recta - This species is comparable to L. siliquoidea in depositional pattern and clarity of microincrement definition.

Strophitus undulatus - The single S. undulatus specimen contained well defined microincrements and several minor growth lines within each macroincrement. This may indicate sensitivity to the environment, coupled with an ability to recover quickly from traumatic events and resume normal deposition of shell.

Chapter 6: COMMENTS ON SAMPLE REQUIREMENTS

No clear indication of the sample size required to make a reasonably accurate estimate of seasonality can be derived from the mussels in this study. The variability in the target population is unknown and cannot be reliably estimated from such a small sample. The variation shown in the deposition of the microincrements, together with the small size of the sample subsets, place severe limits on the confidence which may be accorded to any estimates made from this study sample. In Appendix E the mean ($\bar{X}$), standard deviation (s_x), standard error of the mean ($s_{\bar{x}}$), and sample size estimates (n_o), are calculated for each sample. The first sample is calculated for 95 percent confidence that $\bar{X}$ falls within ± 5 microincrements of μ. The second sample is calculated for a ± 10 error.

The wide variation in these estimates of n_o (1 to 20 for ± 5 1 to 5 for ± 10) indicates that these samples do not afford very great precision in estimating population parameters. In addition, since the samples are not of the same size, they cannot be directly compared, and since the samples were gathered at different times, they cannot be lumped tpgether into a single sample because the variability may be partly a function of the stage of metabolic activity of the population. If the investigator is satisfied with a ± 10 accuracy estimate, a sample of 5 shells might prove to be adequate. A ± 5 level of precision

would require a much larger sample. The averages plotted in Figure 5 indicate that an estimate within a ±10 error could provide an estimate of seasonality of the order of spring, summer or fall within the bounds of 95 percent confidence.

In the Canadian Interior Basin, if it can be assumed that the mussels found in an archaeological site were collected live, an assemblage which shows no indication of the commencement of growth following large macroincrements can probably be assumed to have been gathered late in the season. The harvesting of mussels through a layer of winter ice would be extremely laborious and likely to be highly unproductive. The final sample of seven mussels collected November 26 from station 3 required that a 15 m by 20 m hole be cut, in an increasing radial search for mussels, in an area where earlier in the season they were easily picked from a bed of hundreds. A wet suit, for which there is no documented analogue among aboriginal people in this area, was used to make this collection. Following winter, flooding of river channels occurs in spring prior to, and during breakup of the river ice. It is highly unlikely that mussels would be available to aboriginal populations prior to the commencement of summer deposition of shell material. In the region where the study sample was gathered, it is unlikely that it would prove difficult to distinguish between an assemblage collected in the late fall.

When considering archaeological assemblages, it should

be stressed that provenience of the sample is of the greatest importance. Only those assemblages which the analyst feels confident were excavated under conditions of excellent control, and which represent a discrete collection assemblage, should be used to estimate seasonality. A composite sample will produce a meaningless average, especially if the sample specimens happen as a chance association from different seasons and different years.

CONCLUSIONS

It is clear that the shell growth of freshwater mussels in the Canadian Interior Basin is confined to the period when the waters are ice free, and that maximum growth takes place during the warmest part of the summer. While there is no simple linear relationship to temperature, governing all mussels equally, there is an obvious trend for the average number of microincrements in a sample to increase as the growing season progresses.

The problems associated with observer error, and extreme variation in the rate of deposition of shell material, preclude the use of macroscopic techniques in making seasonality estimates from freshwater mussel shells. There is the additional problem of deciding whether or not a particular macroincrement is an annulus, or if it is representative of some lesser period of time, the boundary of which has been prematurely defined by a hiatus induced by some environmental or physiological event. In microincrement analysis, this uncertainty appears to be mitigated by the averaging procedure which is used following the microscopic counting of the microincrements in an assemblage. While there is a large amount of variability in the rate of microincrement deposition, the laying down of these increments seems to be a much more reliable indication of seasonality than the absolute amount of shell material deposited over a given

period of time. The demonstrated trend in this sample to an increase in the average number of microincrements through time, and subsequent to spring breakup, indicates that seasonality studies on freshwater mussels are feasible. The microscopic examination of stained thin sections appears to be the most satisfactory of the methods of microstructural analysis considered in this study.

The questions of target population variability and required sample size are unresolved and await the collection and analysis of samples gathered with these problems in mind. No attempt has been made to isolate those factors of environment or physiology which might prematurely terminate growth and produce a macroincrement representing a periodicity shorter than a full growth season or annulus. No obvious criteria for making such distinctions were identified during microscopic examination of the shell sections. The positive identification of reliable criteria within the infrastructure of the mussel shells would almost certainly improve the accuracy of seasonal estimates by eliminating some of the artificially truncated or diminished macroincrements from the microincrement averages.

As a final word of caution, it must be stated that since population variability is unknown and since clinal and longitudinal patterns of deposition have not been examined, this thesis does not demonstrate a useable technique for determining seasonality of site occupation from archaeological freshwater mussel shells. It does indicate that the infrastructure of the shells of freshwater mussels records environmental

information from which it may be feasible, using microscopic techniques, to extract data leading to a reasonable estimate of the season during which an assemblage of mussels died. The elucidation of this information will require that studies of population variability, which are reflected in patterns of microincrement deposition, be undertaken on large collections of modern shell for which there is detailed environmental information. It will also prove necessary to conduct these studies across a wide geographical range.

APPENDIX A

MACROSCOPIC OBSERVATION PROCEDURES

Use a random numbers table or percentile dice to select a random sample of 10 specimens from the population of 175 Lampsilis siliquoidea shells. Use the following instructions and the diagram to measure and record the indicated observations. Take all measurements to the nearest 0.5 mm. Make all observations on the left valve.

To take measurements A, B, D, and E, orient the valve in the following manner:

(1) Place the valve on the piece of metric graph paper with the exterior surface up and the umbo along the horizontal scale.

(2) Orient the plane of the hinge parallel to, and touching the horizontal scale.

(3) Maintain this orientation and be certain that the left edge of the shell touches the vertical scale.

(4)Take measurements to the closest half millimeter.

Measurement A (maximum length): With the shell oriented, read the measurement along the horizontal scale as indicated by the extreme right hand edge of the shell. Record this measurement in the appropriate box.

Measurement B (depth from the umbo to ventral edge at right angles to measurement A): With the shell oriented, select the point on the horizontal scale passing through the center of the

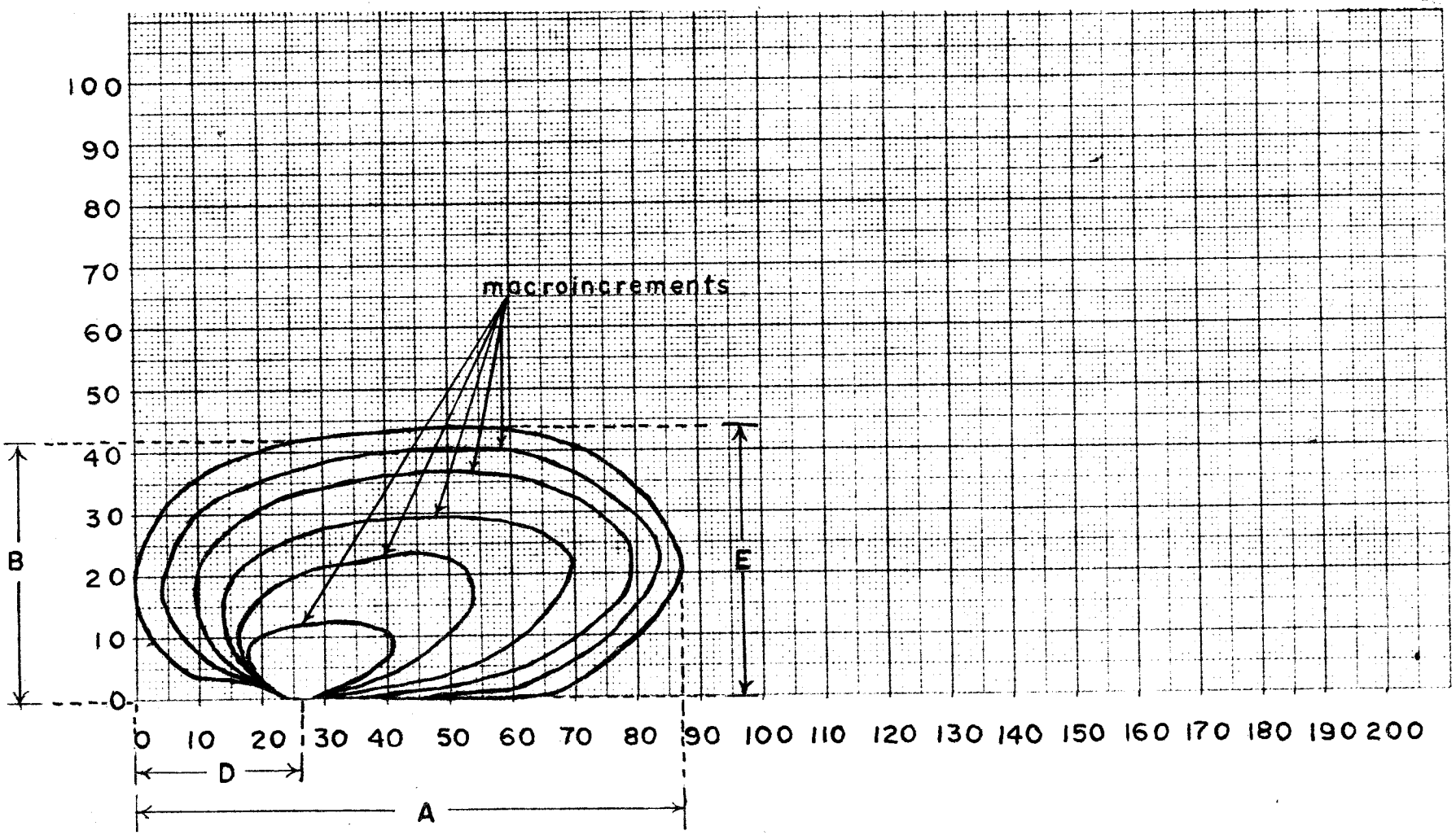
umbo. Read the vertical scale at the point where the ventral edge intersects with the line already selected on the horizontal scale. Record this measurement in the appropriate box.

Measurement C (maximum width of valve): Open the caliper jaws. Place the lower jaw of the spreading caliper posterior to the pseudocardinal tooth and slide the umbo back against the throat of the caliper. Rest the ventral edge and the hinge surface of the shell on the lower jaw of the caliper. Keep the pseudocardinal tooth next to the jaw of the caliper and slide the ventral edge of the shell along the lower jaw while carefully closing the calipers until the point of maximum thickness is found. Read the scale on the calipers and record the measurement in the appropriate box.

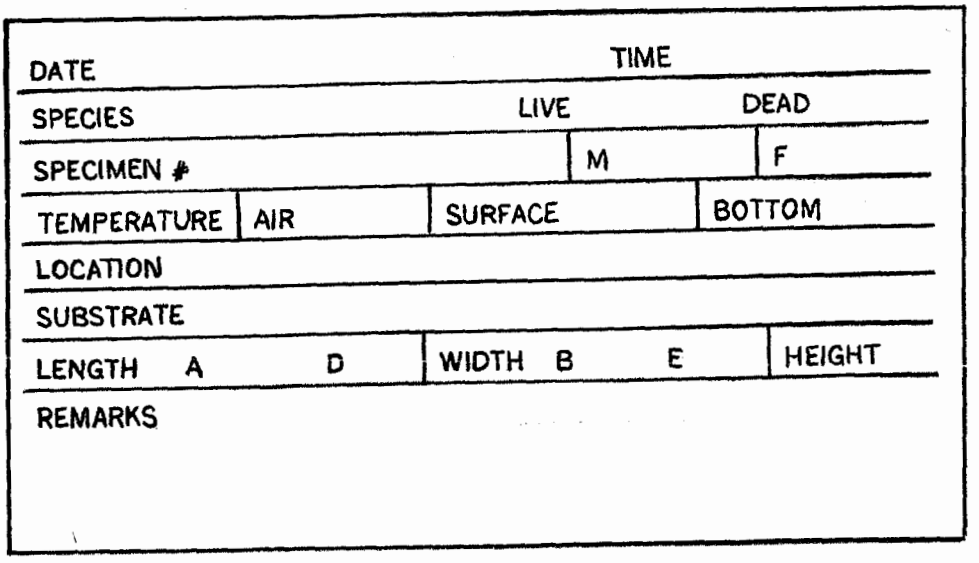
Measurement D (Length from the center of the umbo to the anterior end of the shell measured parallel to the plane of the hinge): With the shell oriented, read the horizontal scale at the center of the umbo and record the measurement in the appropriate box.

Measurement E (Maximum distance from the ventral edge to the plane of the hinge): With the shell oriented, find the point on the shell with the highest value on the vertical scale. Record this value in the appropriate box.

Estimated annuli: Count and record the number of major growth rings on the exterior surface of the shells. Choose criteria such as continuity and relative prominence of the rings. Ignore relative distance between the rings as a criterion; be consistent.



Macroscopic Observations on Freshwater Mussels



Data card for recording Field Information and macroscopic observations.

APPENDIX B

MICROINCREMENT COUNTS OF *L. siliquoidea* COLLECTED AT STATION 3

Slide	M/F	First Increment	Second Increment	Third Increment	Date Collected	Comments
A-001	M	17	85	79	08/07/79	
A-002	M	7	27	78	"	
A-003	M	14	19	61	"	
A-007	F	13	30	13	23/07/79	
A-008	M	12	36	47	"	
A-009	M	9	23	36	"	
A-010	M	16	48	59	"	
A-011	M	16	74	26	"	
A-012	F	16	26	39	08/08/79	Injured
A-013	F	37	82	66	"	
A-014	M	34	68	53	"	
A-015	M	26	12	19	"	
A-016	M	35	47	58	"	
A-017	M	32	95	45	"	
A-018	M	36	27	32	"	
A-021	M	58	16	66	17/08/79	
A-022	F	69	105	95	"	
A-023	F	68	142	60	"	
A-024	F	66	85	65	"	

A-025	F	77	51	--	17/08/79	
A-026	F	64	57	78	"	
A-027	F	56	63	90	"	
A-028	M	45	64	43	"	
A-029	M	27	26	39	"	
A-030	F	33	88	52	"	
A-031	M	48	76	122	29/08/79	
A-032	M	43	65	73	"	
A-033	M	53	78	64	"	
A-034	M	59	102	109	"	
A-035	M	35	34	37	"	
A-036	M	79	--	91	"	
A-038	-	123	76	44	"	
A-045	F	92	105	73	28/09/79	
A-046	F	76	71	85	"	
A-047	F	110	59	77	"	
A-048	F	56	83	37	"	
A-049	F	46	117	115	"	
A-050	F	92	121	61	"	
A-051	F	60	42	68	"	
A-052	F	27	77	50	"	Lesion
A-053	M	66	--	--	"	
A-054	M	22	38	44	"	Lesion
A-069	F	84	98	59	26/11/79	
A-070	F	76	127	63	"	
A-071	F	68	147	55	"	
A-072	F	89	68	108	"	

A-073	F	79	104	96	26/11/79
A-074	F	85	101	90	"

APPENDIX C

PREPARATIONS USED IN LAB PROCEDURES

Formol Saline Solution - This solution is prepared from a mixture of 900 ml distilled water, 100 ml of 40 percent commercial formaldehyde concentrate and 9 gms of sodium chloride.

Toluidine Blue O Stain - This solution is prepared from 200 ml distilled water and 1 gm Toluidine Blue O powder (500 ppm).

Alizarin Red S Stain - This solution is prepared from 200 ml distilled water and 1 gm Alizarin Red S powder (500 ppm).

FLO-TEXX - This is a commercial mounting medium or liquid cover slip manufactured by Lerner Laboratories, Stamford, Connecticut. It contains an oxidizing agent to help prevent stains from fading.

Hillquist Lapidary Epoxies - These are commercial epoxies used for mounting petrographic specimens. The A/B combination was found satisfactory for mounting the unstained shell, but the C/D cover slip mount extracted the stain from the thin sections and diffused it over the entire section.

APPENDIX D

GLOSSARY OF KEY TERMS

Conchiolin - Albuminoid organic matrix of a mollusc shell

Ectostracum - Prismatic outer layer of palliostracum

Endostracum - Innermost nacreous layer of a bivalve shell

Growth Line - Any line observable in the infrastructure of a bivalve shell marking an interruption of continuous shell deposition (i.e. diurnal, annulus)

Growth Ring - Major line on the exterior of a bivalve shell believed to represent a major event in the life of the animal (i.e. annulus)

Hiatus - Cessation of growth due to environmental, biological traumatic random events

Macroincrement - A major segment of the infrastructure of a bivalve shell bounded by major growth lines and which is continuous throughout the palliostracum; believed to represent some large scale periodicity (i.e. annulus)

Mantle - Integument that surrounds vital organs of a mollusc and secretes shell

Mesostracum - Nacreous inner layer of palliostracum

Microincrement - The small scale segments in the infrastructure of a bivalve shell; bounded by minor growth lines in the

prismatic layer of the Unionidae and believed to represent a small scale periodicity (i.e. diurnal)

Myostracum - Deposits of the pallial line and adductor muscles

Nacreous - Type of shell structure consisting of thin tabular crystals of aragonite lying parallel to the growth lines.

Pallial Line - Line or narrow band on interior of valve close to margin and marking the line of attachment of the marginal muscles of the mantle

Palliostracum - Outermost layer of bivalve shell composed of prismatic ectostracum and nacreous mesostracum

Periostracum - Thin layer of horny material (conchiolin) covering the calcareous exterior of a bivalve shell

Prismatic Layer - Type of shell structure consisting of prisms of calcite or aragonite perpendicular to the growth lines

Radial - Direction of growth outward from the beak (umbo) at any point on the shell

Umbo - Region of valve surrounding point of maximum curvature of longitudinal dorsal profile (synonymous with the beak in Lampsilis)

APPENDIX E

STATISTICAL VALUES FOR SAMPLES COLLECTED AT STATION 3

FIRST INCREMENT COUNTS

Mean $\bar{X} = \Sigma X_i / n$

Standard Deviation $s_X = \sqrt{\frac{\Sigma(X-\bar{X})^2}{n-1}}$

Standard Error of the Mean $s_{\bar{X}} = s_X / \sqrt{n}$

Sample size required for confidence interval ± 5; 95% confidence limit. Bracketed figure () for ± 10 limit.

$$n_o = \left[\frac{1.96(s_X)}{5} \right]^2$$

July 8	$n = 3$	$s_{\bar{X}} = 2.96$
	$\bar{X} = 12.67$	$n_o = 4$
	$s_X = 5.13$	$= (1)$
July 23	$n = 5$	$s_{\bar{X}} = 1.32$
	$\bar{X} = 13.2$	$n_o = 1$
	$s_X = 2.95$	$= (1)$
Aug. 8	$n = 6$	$s_{\bar{X}} = 7.11$
	$\bar{X} = 45.5$	$n_o = 47$
	$s_X = 17.42$	$= (12)$

Aug. 17	$n = 10$	$s_{\bar{X}} = 5.18$
	$\bar{X} = 56.3$	$n_o = 41$
	$s_X = 16.37$	$= (10)$
Aug. 29	$n = 7$	$s_{\bar{X}} = 11.32$
	$\bar{X} = 62.86$	$n_o = 138$
	$s_X = 29.95$	$= (34)$
Sept.28	$n = 8$	$s_{\bar{X}} = 7.69$
	$\bar{X} = 74.75$	$n_o = 73$
	$s_X = 21.75$	$= (18)$
Nov. 26	$n = 6$	$s_{\bar{X}} = 3.07$
	$\bar{X} = 80.17$	$n_o = 9$
	$s_X = 7.52$	$= (2)$

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