GREAT LAKES FISHERY COMMISSION

Project Completion Report[1]

The use of calcareous otic elements (statoliths) to determine the age of sea lamprey ammocoetes (Petromyzon marinus)

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THE USE OF CALCAREOUS OTIC ELEMENTS (STATOLITHS) TO DETERMINE THE AGE OF SEA LAMPREY AMMOCOETES (*Petromyzon marinus*).

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INTRODUCTION

During the long history of fisheries management efforts aimed at the erradication of the sea lamprey (Petromyzon marinus) from The Great Lakes, biologists have had to rely upon analysis of length frequency data from large ammocoete collections for critical information about population age structure and growth rates of the ammocoete larvae. Unfortunately, the reliability of the technique is severely limited in all but the youngest fish due to slower and more variable growth rates in older fish resulting in considerable overlap of length distributions corresponding to year classes (Potter 1980).

Despite these shortcomings, there has been no alternative to the use of length frequency analysis to age lampreys because although scales and otoliths have proved invaluable for ageing teleost species, lampreys have no scales and their calcareous otic elements (statoliths) have gone uninvestigated in this regard (Potter 1980). As early as 1912, Studnicka described statoliths in the membranous labyrinth of the sea lamprey and in the statoliths of the river lamprey (Lampetra fluviatilis) Carlstrom (1963) noticed internal banding patterns. Volk and Brothers (ms) noticed similar banding patterns in the statoliths of sea lamprey ammocoetes in Cayuga Lake, N.Y. and showed a positive relationship between statolith band number and ammocoete size.

It is the purpose of this research to investigate the hypothesis that bands are produced annually in the statoliths of sea lamprey ammocoetes and thereby represent a tool for directly ageing these fish.

RESULTS

Mean total ammocoete length and mean statolith diameter measured on three axes (fig. 1) are presented for each collection in table 1. Among all collections combined, there was an allometric relationship between statolith size and total ammocoete length, regardless of which axis was used for statolith measurement (For A_1, $y=20.78\ x^{.55}$, $r=.98$; for A_2, $y=58.97\ x^{.32}$, $r=.97$; for A_3, $y=31.67\ x^{.28}$, $r=.96$: where y is mean statolith size and x is mean fish size for each ammocoete collection). In a test utilizing samples from four ammocoete age classes, there was no significant difference between the size of right and left statoliths for all axes of statolith measurement (t test, $p > .05$; app. 1). All subsequent statolith measurements were from the left statolith.

When a statolith in immersion oil was oriented properly to transmitted incident light, numerous bands appeared as layers within the statolith (fig. 2). Two to four very thin bands were present near the apex of most statoliths (fig. 2a) and their presence even in yearling specimens indicates a sub-annual periodicity. These bands were easily distinguished from other very dark and obvious bands present in nearly all statoliths (fig.2 a,b,c). Excepting only those few statoliths which showed none of these dark bands (table 2), one was invariably formed at the statolith base. It was my hypothesis that these dark statolith bands were produced on an annual basis.

The results of dark band counts in ammocoete statoliths are presented in table 2. Collections of age 1 fish from The Brule and Ford (sec. 2 & 11) Rivers revealed statoliths almost exclusively with one band, however, those from The Carp and Ford (sec. 31) Rivers were divided between those whose statoliths showed one band and those having no major statolith bands. For those fish determined to be age 2,3,or 4 years, statolith band number was in agreement with those ages in over 90% of readable statoliths for every collection except that from The Days River where one of five specimens had three bands. Age 7 specimens from The Little Gratiot River had statolith band counts ranging from 5 to 7 and statoliths of unknown age specimens from

The Big Garlic and Oswego Rivers had from 4 to 11 bands (table 2). Ammocoetes from The Pine River had either very small statoliths apparently just forming or none at all.

The number of ambiguous or unreadable statoliths ranged from 0% to 44% among all collections (table 2). When both statoliths were readable, there was never a discrepancy between right and left members.

In order to evaluate the reliability of statolith band counts, a sample from each ammocoete collection was counted a second time. Agreement was precise between the two counts in all but four samples (app. 2). In the case of one year old fish from The Ford River (sec.31) and two year old fish from Nebagemon Creek, just one statolith was counted differently in the two trials. Although the two frequency distributions agree well between trials for unknown age fish from The Oswego River, in reality only 33% of statoliths had similar band counts in the two trials and the match up of band count frequencies is the result of offsetting differences in successive band counts. Only 16.7% of statolith band counts for ammocoetes from The Big Garlic River were in agreement between trials.

Sixteen specimens were viewed with the scanning electron microscope (SEM) to determine if internal banding patterns manifest themselves on the statolith surface. Figure 3 shows examples from three specimens of different ages and it is quite clear that discontinuities in the form of bands around the perimeter of the statoliths are present. Age 1 ammocoetes examined showed no such bands while the four age two ammocoetes exhibited a single discontinuity in the statolith between apex and base (fig. 3a). Age 4 specimens and transforming ammocoetes of unknown age also had one major band near the apex, however, subsequent bands were present in varying number and were difficult to discern (fig 3 b,c).

DISCUSSION

The results of this research show clearly that for sea lamprey ammocoetes aged one to four years, statolith band number is a very reliable indicator of fish age. For fish two to four years old, there are very few specimens in which statolith band number does not agree with the age determined by Great Lakes Fishery Commission personel. However, two collections of age one specimens show larger discrepancies, where statolith band counts are split almost evenly between those with one band and those with none. Because it is virtually certain that these specimens are indeed yearling fish, the explanation for this is either that these few fish failed to form their first annulus or that this annulus would have formed shortly after collection. Although I cannot distinguish between the two possibilities with certainty, it seems very unlikely that these fish would be the only ones not to form a first annulus. Furthermore, the fact that all other statoliths examined showed their ultimate annulus directly at their base indicates a fairly recent formation of the band as little statolith growth has occurred since. Also, it is known that annuli in otoliths of temperate teleosts form during the winter and spring months prior to the onset of the next growing season (Bagenal 1974). Thus, there is reason to believe that the first statolith annulus is incipient in these age one specimens, but, in any case, this incongruence does not offer serious difficulties for our ability to age them.

In the small number of age seven specimens examined, the relationship between statolith band number and fish age is not very rigorous, with only one specimen showing seven statolith bands. The most likely explanation for this poor relationship lies with ontogenetic changes in fish growth rate and the allometric relationship between statolith size and fish size. In short, the equations which describe this relationship (see results) show that the growth rate of statoliths falls exponentially along all axes as ammocoetes get larger and older. The end result of this is that statolith bands produced on an annual basis will be ever closer together and increasingly difficult to resolve. This problem of reader inability to distinguish statolith bands in older fish is also reflected in the poor agreement between successive statolith band counts in unknown age fish with four to eleven statolith bands. Although this is clearly a general problem with older fish, its magnitude will depend upon the actual growth rate of the fish, the task being theoretically easier where fish maintain somewhat higher growth rates. It was hoped that the problem might be mitigated somewhat through the use of SEM with the possibilty that banding patterns might be obvious externally at high magnifications, however, figure 3 indicates that such patterns are difficult to decipher on the statolith surface.

In summary, this research documents the feasability of directly determining the age of sea lamprey ammocoetes through annual bands produced in their statoliths. The advent of this method marks a distinct improvement over the use of length-frequency data to age ammocoetes as it permits the assignment of ages to individual fish with reliability. This, in turn, will allow the assessment of individual variability in population age structure and growth rates as well as the ability to assess age at transformation for some ammocoete populations.

However, although the reliability of this technique is excellent for fish up to four years of age, the limits of its application beyond this age have not been defined by this study. The only indications I have in this regard are from ten age seven specimens and general conclusions based on such a small sample should remain tenuous.Future research should seek a more extensive collection of known age ammocoetes older than four years to determine the limits of this method and adult lampreys should be examined for banding patterns in their statoliths as well.

One of the most important applications of this methodology is the determination of age structure in older and transforming ammocoete populations. While I have shown that the assignment of individual ages to fish older than six years is dubious, it should be pointed out that in the unknown age ammocoete collections from The Big Garlic and Oswego Rivers , while repeat statolith band counts showed poor agreement, the mean statolith abnd numbers between the two trials for each collection were not significantly different (t test; $p > .05$). Mean band number was significantly different between the two collections ($p < .05$). This points out that age comparisons based on population samples rather than individuals can still provide a great deal more information about these older ammocoetes, including the potential to determine age at transformation, than length frequency has been able to. Attention should be directed to assessing the variability in statolith band number in these older and transforming age classes of ammocoetes.

LITERATURE CITED

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Table 1. Mean total ammocoete length and mean statolith diameter measured on three axes (A_1,A_2,A_3; see fig. 1) for each ammocoete collection. Numbers in parentheses represent one standard error. Sample sizes for fish length (N_f) and Statolith diameter (N_s) are also given.

Collection Location	Ammocoete Age	Mean Ammocoete Length (mm.)	Mean Statolith Diameter (μm.) A_1	A_2	A_3	N_f	N_s
Ford R.(sec. 31)	1	30.56 (0.54)	145.80 (3.18)	176.33 (2.17)	86.43 (0.89)	29	26
Ford R.(sec. 2&11)	1	29.24 (0.57)	130.32 (3.26)	173.16 (1.97)	88.08 (1.05)	30	15
Brule R.	1	33.72 (0.76)	140.76 (2.62)	181.26 (2.29)	87.48 (0.90)	30	29
Carp R.	1	31.15 (0.70)	132.78 (1.65)	171.66 (2.43)	82.39 (1.14)	30	30
Ford R.(sec. 31)	2	66.11 (1.16)	209.85 (4.23)	223.13 (3.01)	107.00 (0.83)	30	27
Ford R.(sec.2&11)	2	62.23 (1.34)	211.40 (3.06)	217.22 (3.31)	107.68 (1.06)	30	28
Trout Cr.	2	48.94 (1.90)	163.69 (3.05)	198.90 (3.43)	88.65 (1.14)	18	16
Millecoquins R.	2	43.94 (0.85)	167.04 (2.53)	199.05 (2.62)	90.51 (0.75)	30	30
Albany Cr.(sec. 20&29)	2	50.17 (0.76)	166.34 (2.72)	196.50 (2.05)	90.62 (1.28)	30	30
Nebagemon Cr.	2	70.11 (1.14)	215.99 (3.47)	224.58 (3.43)	104.75 (1.11)	27	27
Albany Cr.(sec. 5)	2	44.36 (0.97)	175.86 (2.25)	205.10 (2.37)	90.68 (0.72)	29	29
Steeles Cr.	2	54.27 (1.82)	195.45 (3.02)	214.32 (2.90)	94.80 (0.91)	15	15
McKay Cr.	2	44.94 (2.45)	156.15 (6.98)	186.78 (5.80)	84.15 (2.80)	10	8
McKay Cr.	3	81.00 (3.44)	219.60 (9.34)	240.84 (8.62)	107.64 (0.36)	5	5
Betsy R.	4	115.49 (1.90)	276.77 (3.56)	255.43 (3.89)	123.14 (1.99)	30	29
Days R.	4	125.30 (2.99)	286.20 (6.61)	262.44 (4.27)	125.28 (2.39)	5	5
Pt. Patterson Cr.	4	105.15 (1.87)	278.10 (3.47)	258.90 (2.63)	123.06 (1.12)	30	30
Little Garlic R.	4	112.98 (0.99)	289.32 (2.74)	279.55 (3.13)	122.10 (1.21)	30	30
Little Gratiot R.	7	160.07 (3.21)	284.76 (6.99)	272.88 (2.28)	127.26 (1.40)	10	10
Oswego R.	UNK.	130.00 (2.70)	335.62 (5.57)	299.29 (7.09)	128.70 (1.99)	25	12
Big Garlic R.	UNK.	128.72 (2.51)	320.58 (4.76)	283.98 (4.82)	120.06 (1.11)	30	25

Table 2. Frequency distributions of statolith band number and ambiguous or non-readable (A/NR) statoliths for each ammocoete collection. Sample size is also indicated (N).

Ammocoete age	Location	Band Number 0	1	2	3	4	5	6	7	8	9	10	11	A/NR	N	
1	Ford River	.34	.52											.14	29	(sec. 31)
1	Ford River		.93											.07	15	(sec. 2 & 11)
1	Brule River	.03	.79											.18	29	
1	Carp River	.50	.47											.03	30	
2	Ford River			.96										.04	27	(sec. 31)
2	Ford River			.93										.07	28	(sec. 2 & 11)
2	Trout Cr.			1.00										.00	16	
2	Millecoquins R.			.90	.10									.00	30	
2	Albany Cr.		.04	.69										.27	26	(sec. 20 & 29)
2	Nebagemon Cr.			.93										.07	27	
2	Albany Cr.			.93	.07									.00	29	(sec. 5)
2	Steeles Cr.			.93										.07	15	
2	McKay Cr.			.80										.20	10	
3	McKay Cr.				1.00									.00	5	
4	Betsy River					.97								.03	30	
4	Days River				.20	.80								.00	5	
4	Pt. Patterson Cr.					1.00								.00	30	
4	Little Garlic R.				.03	.87	.07							.03	30	
7	Little Gratiot R.						.22	.44	.11					.22	9	
UNK.	Oswego River					.17	.50	.17	.08					.08	12	
UNK.	Big Garlic R.					.02	.22	.15	.02	.10	.02		.02	.44	41	

Figure 1. Scanning electron micrographs of statoliths from four-year old sea lamprey ammocoetes (250 x). A.) Photograph showing the proper spatial orientation of statoliths. B.) Axes of statolith measurements: A_1 axis runs perpendicular to the anterior-posterior axis intersecting the apex of the statolith. A_2 axis is the anterior-posterior axis, also intersecting the apex. A_3 (not drawn in) is measured from the apex directly down to the base plane while the statolith is propped on edge.

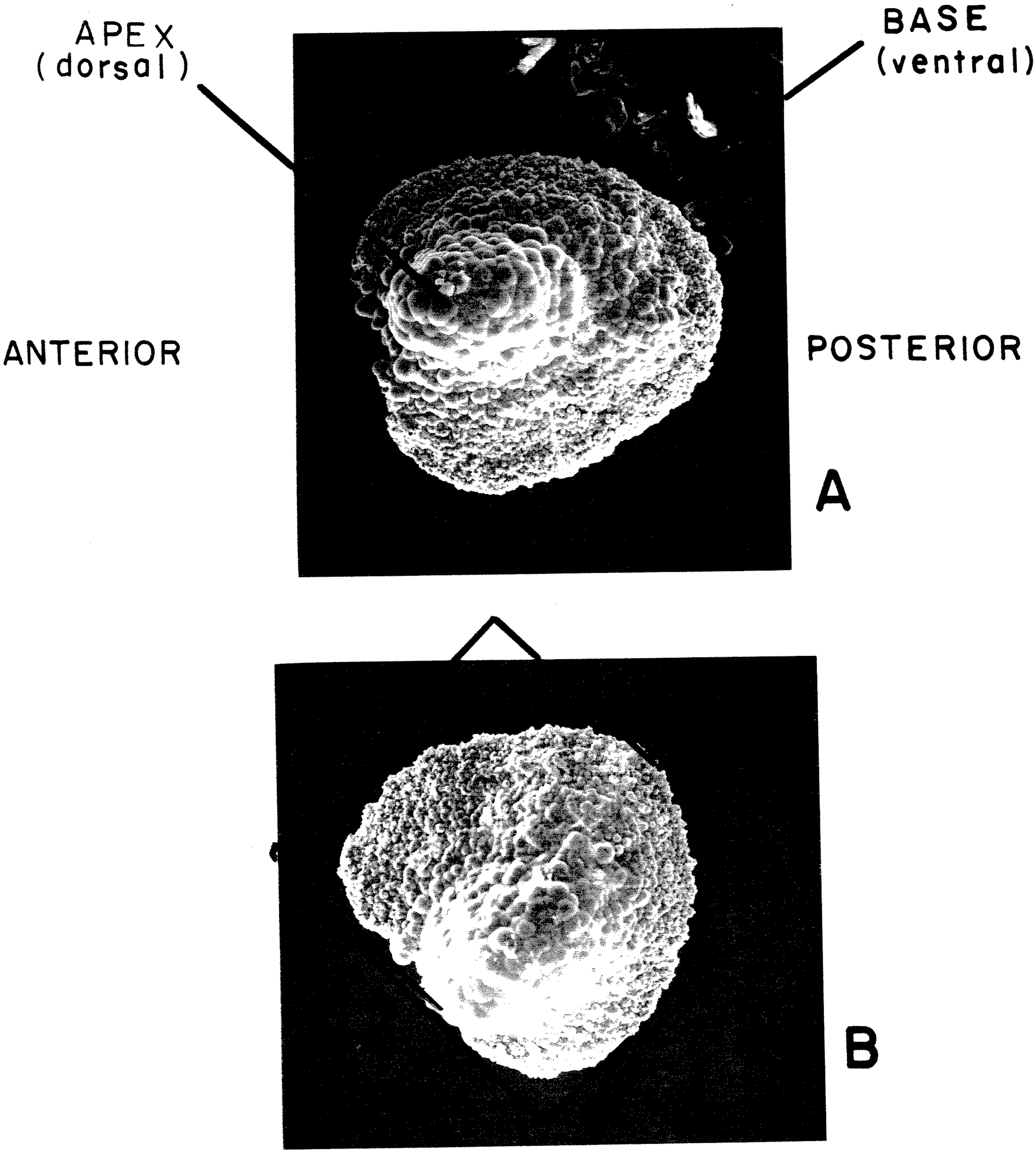

APEX
(dorsal)
BASE
(ventral)
ANTERIOR
POSTERIOR
A
B

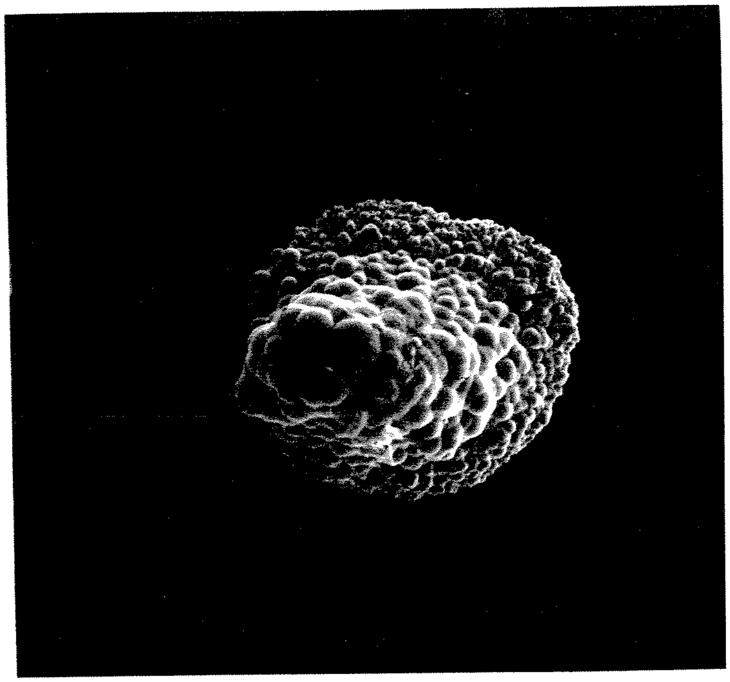
Figure 2. Statoliths photographed through a dissecting microscope at 250 x using transmitted light. A.) Statolith from a one year old ammocoete (31.42 mm.)showing the first annulus directly at the base and several sub-annual bands near the apex. B.) Statolith from a two year old ammocoete (49.21 mm.) showing two annuli and a sub-annual band just above the second annulus. C.) Statolith from a four year old ammocoete (116.45mm.) showing four annuli. Annuli are denoted with small,black checks.

A

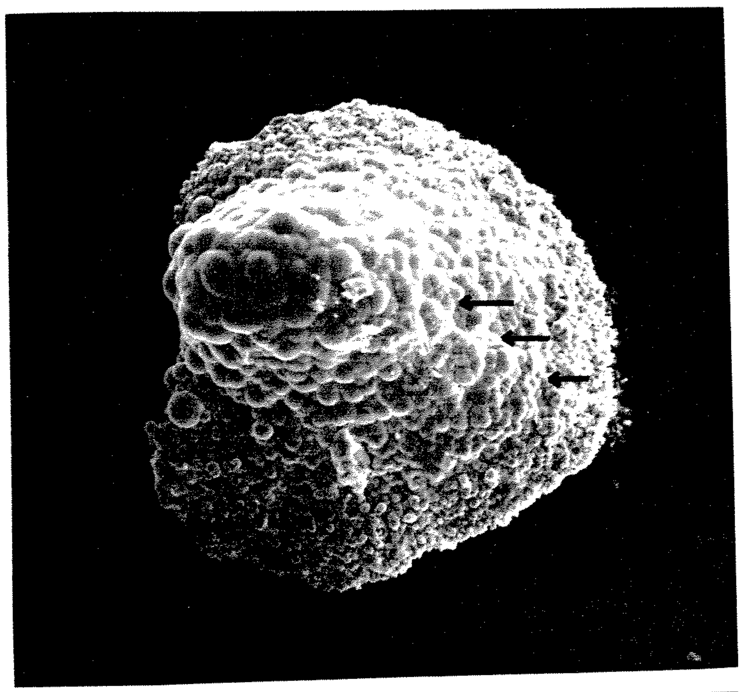
B

C

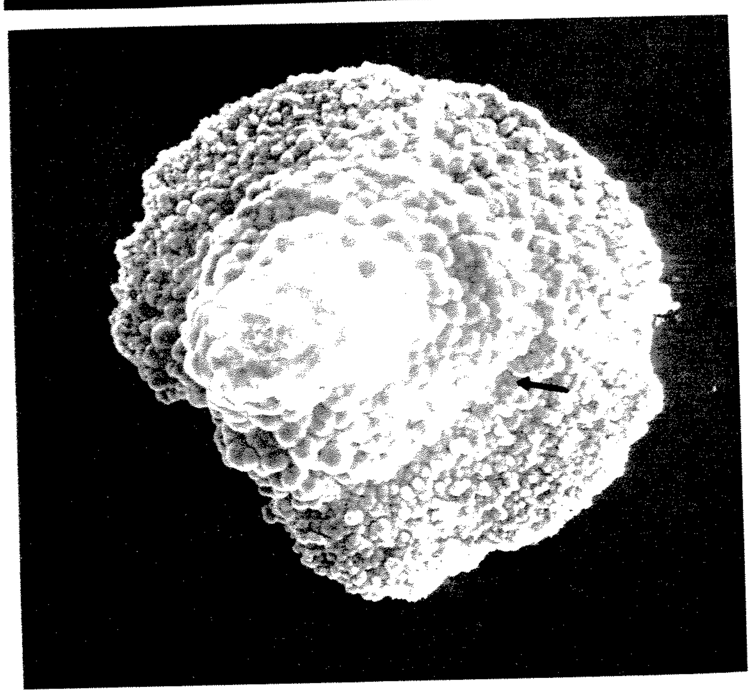
Figure 3. Scanning electron micrographs of ammocoete statoliths. A.) Statolith from a two year old ammocoete (60.40 mm.), B.) Statolith from a four year old ammocoete (115.50 mm.) C.) Statolith from an unknown age ammocoete just beginning to transform (130.60 mm.). All magnifications are 250x. The statoliths exhibit distinct discontinuities in the form of bands on the external surface which may correspond to internal bands seen with light microscopy. These are denoted with arrows.



A



B



C

Appendix 1. Values for the t statistic to test the hypothesis that mean statolith size, measured on any axis, is the same for right and left members. There is no significant difference between the means in all cases. Numbers in parentheses are degrees of freedom.

Collection Location	Ammocoete Age	t values for comparison of right and left mean statolith size on each measurement axis.		
		A_1	A_2	A_3
Brule River	1	1.23(54)	0.93(53)	-0.45(56)
Millecoquins R.	2	-0.88(66)	-1.25(67)	-1.02(68)
Betsy River	4	-0.63(43)	-0.84(49)	-0.70(50)
Little Gratiot R.	7	-0.99(12)	-1.42(12)	0.09(12)

Appendix 2. Comparison of statolith band number frequencies for counts made on two seperate occassions in samples from each ammocoete collection. Data for each trial is represented by a number or numbers in the two horizontal rows for each ammocoete collection.

Collection Location	Ammocoete Age	Statolith Band Number 0	1	2	3	4	5	6	7	8	9	10	11	N
Ford R.(sec. 31)	1	.29	.71											7
		.14	.86											
Ford R.(sec.2&11)	1		1.00											6
			1.00											
Brule R.	1		1.00											10
			1.00											
Carp R.	1	.38	.62											8
		.38	.62											
Ford R.(sec.31)	2			1.00										10
				1.00										
Ford R.(sec.2&11)	2			1.00										14
				1.00										
Trout Cr.	2			1.00										10
				1.00										
Millecoquins R.	2			1.00										12
				1.00										
Albany Cr. (sec.20&29)	2		.20	.80										5
			.20	.80										
Nebagemon Cr.	2			1.00										10
				.90	.10									
Albany Cr.(sec.5)	2			.90	.10									10
				.90	.10									
Steeles Cr.	2			1.00										10
				1.00										

Appendix 2(cont.)

Collection Location	Ammocoete Age	0	1	2	3	4	5	6	7	8	9	10	11	N
McKay Cr.	2			1.00										6
				1.00										
McKay Cr.	3				1.00									5
					1.00									
Betsy R.	4					1.00								21
						1.00								
Days R.	4				.20	.80								5
					.20	.80								
Pt. Patt. Cr.	4					1.00								10
						1.00								
Little Garlic R.	4					1.00								10
						1.00								
Little Gratiot R.	7						.67	.33						3
							.67	.33						
Oswego R.	UNK.					.18	.55	.18	.09					11
						.09	.55	.18	.09	.09				
Big Garlic R.	UNK.						.33	.17	.17	.17	.08		.08	12
							.08	.08	.58	.08	.17			