GREAT LAKES FISHERY COMMISSION
Research Completion Report*

USE OF STATOLITHS TO VALIDATE THE AGE-COMPOSITION OF SEA LAMPREY POPULATIONS ESTIMATED FROM LENGTH-FREQUENCY DISTRIBUTIONS

by

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Abstract

Statolith banding patterns in larval sea lamprey were examined to validate age composition estimated from length-frequency distributions. The larvae were collected from six southern Ontario tributaries treated with lampricide in 1989. There was good agreement with respect to the number of age-groups identified by both aging methods and the expected age-composition based on lampricide treatment history. Statoliths identified residual larvae in three populations (Bronte, Salem, and Cobourg Creeks) which were not apparent in length-frequency analysis. Statoliths also indentified an age-group (3+) in Cobourg Creek that was expected, but not distinguishable in the length-frequency distribution for this population. Mean age-group lengths estimated by each method were similar below approximately 90 mm. At large sizes, length-frequency analysis produced higher estimates of mean length than obtained were from statoliths. Further work is necessary to determine how much of these deviations is real and how much is related to the small number of large individuals aged by statoliths. These results provide tentative validation of ages and sizes of age-groups estimated from length-frequency distributions of larval populations in tributaries regularly treated with lampricide.

Introduction

Accurate ages are fundamental to an understanding of the ecology and biology of fish populations. The absence of hard body parts such as scales and bones has hampered the estimation of life-history parameters in larval lamprey populations. Because lamprey in temperate regions spawn during a short period in each year, polymodal size frequency distributions have been used to distinguish modal size (age) groups from which growth and mortality can be estimated. The results are satisfactory when applied to young, fast-growing age groups, but the slower, more variable growth rates in older animals result in the overlapping of lengths corresponding to age-classes (Potter 1980).

The performance of size-frequency analysis must be validated in each study before size-frequency data can be accepted to reflect population structure. France et al. (1991) described three validation procedures for crayfish studies which are also applicable to lampreys: (1) temporal sampling replication before and after the non-growth period (winter) to measure the precision of data collection and size-frequency analysis; (2) verification with known-age animals from either field recaptures or laboratory studies; and (3) concordance with independent analyses of age composition. To the best of my knowledge there are no published studies documenting the former two procedures over all age-groups of larval lamprey. Age determination from statolith bands is a direct method of aging larval lamprey, independent of their size, and therefore can be used to validate length-frequency analyses.

A statolith is a calcareous structure overlying the otic macula in lampreys (Carlstrom 1963) and squid (Rodhouse and Hatfield 1990) and is functionally analogous to an otolith in teleosts. Each labyrinth contains several statoliths, of which the largest is known to exhibit a distinct pattern of internal banding (Carlstrom 1963). Volk (1986) reported a positive correlation between the number of internal bands and total length for larval sea lamprey, *Petromyzon marinus*, from the Great Lakes. Statolith bands were subsequently validated as year marks or annuli in mountain brook lamprey, *Ichthyomyzon greeleyi* (Medland and Beamish 1987), sea lamprey and American brook lamprey, *Lampetra appendix* (Beamish and Medland 1988), to 5+ yr of age. Statolith growth is directly correlated with environmental temperature above a threshold temperature between 8 and 12 °C, indicating that annulus formation occurs during the winter in individuals that experience strong seasonal growth patterns (Medland and Beamish 1991).

Accurate knowledge of the age structure of larval sea lamprey populations in Great Lakes tributaries is important as the control program is designed to treat natal streams with selective lampricides before the larvae metamorphose and migrate into the Great Lakes (Smith et al. 1974). Treatment schedules are set approximately one year in advance based on samples of larvae and growth rates inferred from length-frequency distributions. Age determination from statoliths has not been widely applied to these populations because the technique has only recently been developed and validated. This study has three objectives: (1) to evaluate the general applicability of statolith aging in sea lamprey populations in tributaries regularly treated with lampricide; (2) to provide information on the age structure of sea lamprey in those streams; and (3) to evaluate the accuracy of the lengths and number of age-groups estimated using length-frequency analysis by comparing them with the results of age determination using statolith bands.

Methods

A random sample of all size ranges of sea lamprey larvae was collected from six southern Ontario streams (Figure 1) during lampricide treatments in the spring and late summer of 1989 (Table 1). Larvae were also collected during lampricide treatments of Farewell Creek (Lake Ontario) and Big Creek (Lake Erie) but were not used in this study because sample sizes were less than 200 (see below). All collections were made from stream sections in which abundance was greatest based on previous treatment data. Transforming larvae were collected from tributaries treated in August, but were excluded from any analysis.

Total lengths (TL) were measured to the nearest mm in the field before larvae were frozen (-20 °C) for later aging in the laboratory. Frozen larvae were subsampled (N = 50 for all populations) and a pair of statoliths were removed, stored in glycerol for 130-179 days between October 1989 and June 1990 and then mounted on slides using an acetone soluble crystalbond adhesive. When more than one statolith was found in either otic capsule, the largest statolith was used. The initial subsampling of larvae from two populations was unsuccessful due to unforeseen difficulties with the protocol; more were removed during July and August 1991 from ammocoetes collected in Bowmanville (N = 56) and Salem (N = 50)

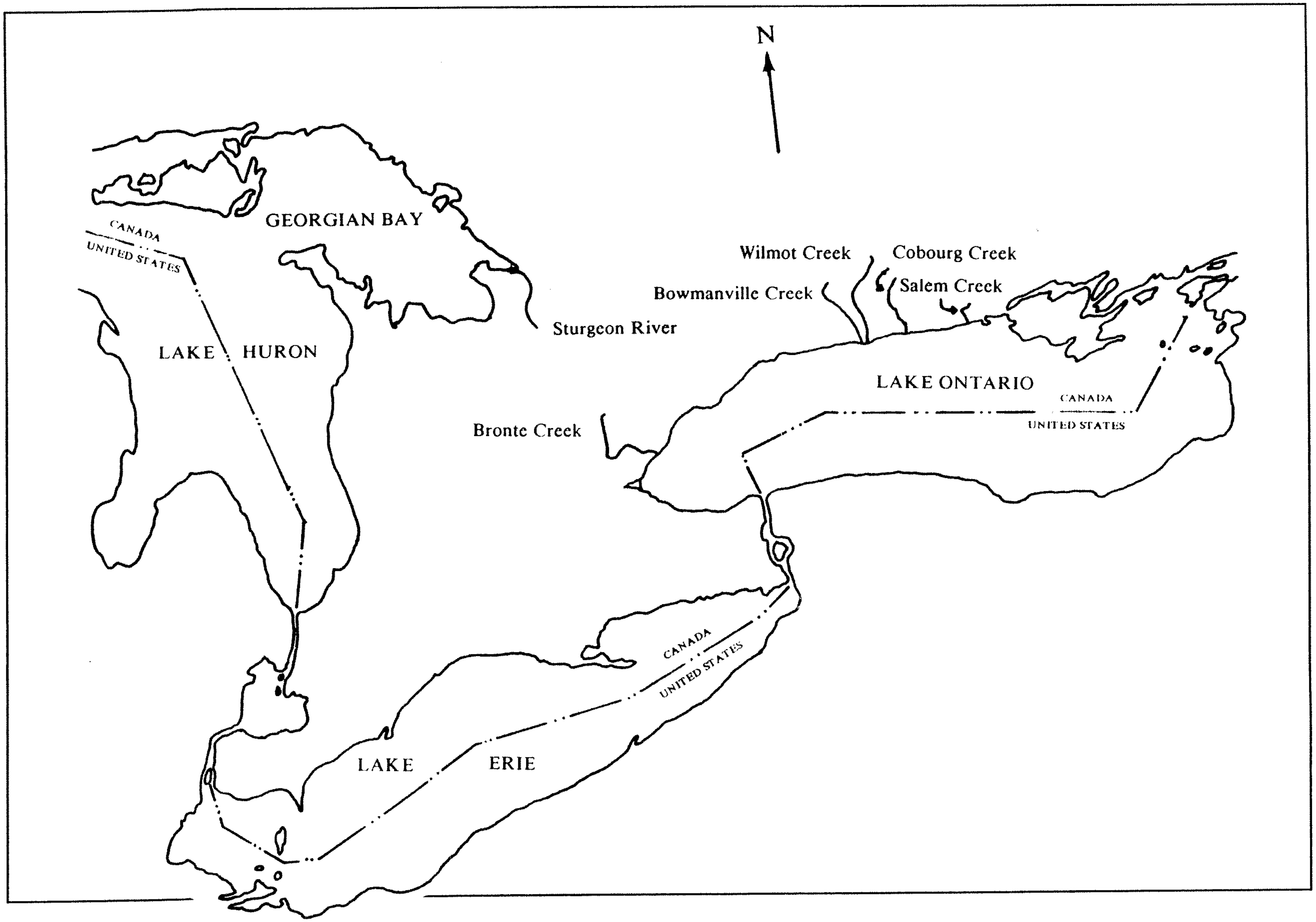

Figure 1. Location of tributaries from which larval sea lamprey were sampled during 1989 lampricide treatments.

Table 1. Collection and length data of larval sea lamprey populations that were aged in this study.

Stream	Collection date	Number collected	Length range (mm)	Number aged	Length range aged (mm)
Bowmanville Creek	Apr 27, 1989	251	30-158	30[a]	42-90[b]
Bronte Creek	May 4, 1989	484	30-167	21[c]	35-151
Sturgeon River	Aug 22, 1989	242	17-170	50	18-133
Salem Creek	Aug 24, 1989	561	15-155	50[a]	20-135
Wilmot Creek	Aug 26, 1989	203	18-142	50	18-134
Cobourg Creek	Aug 29, 1989	182	23-155	45	55-149

[a] Data are from statoliths removed from the second subsample of larvae in July and August 1991.

[b] Not all statoliths from ammocoetes > 90 mm survived the preparation procedure sufficiently intact to identify bands.

[c] Statoliths were removed from 50 larvae, but the majority did not survive the preparation procedure sufficiently intact to identify bands.

Creeks and stored in glycerol for 33 and 14 days respectively. The length range of larvae from which statoliths were removed was similar to the range of lengths collected during lampricide treatments (Table 1). Because this subsampling was not random, mean and median lengths of age-groups were calculated and compared.

There is no standardized protocol for preparing and storing statoliths removed from lamprey larvae. Other researchers have stored statoliths in glycerin at -20 °C and in immersion oil for periods ranging from a minimum of 15 days to 30 days (Volk 1986; Medland and Beamish 1987, 1991; Beamish and Medland 1988). None of these studies reported problems determining age, regardless of the protocol that was followed. On this basis and for logistic reasons, I used a different storage medium and longer storage periods than previously used.

Each pair of statoliths was numerically encoded so that age estimates would not be biased by knowledge of length of the individual from which the statoliths were removed, or by previously assigned ages (Beamish and Medland 1988). The dark bands on statoliths were counted three times with a light microscope (160X magnification) using transmitted light. Only complete major bands were counted; incomplete minor bands, which were observed between major bands, were ignored. Approximate age was equivalent to the number of bands; more exact aging was not attempted because I did not collect information on hatching dates. The precision of the counting technique (in %) was estimated using the following index of average error:

$$\frac{1}{N}\sum_{j=1}^{N}\left[\frac{1}{R}\sum_{i=1}^{R}\frac{|X_{ij}-X_j|}{X_j}\right] x\ 100$$

where N is the number of fish aged, R is the number of times each fish is aged, X_{ij} is the *i*th age determination of the *j*th fish, and X_j is the average age calculated for the *j*th fish (Chilton and Beamish 1982). The lengths and annulus counts of aged larvae are shown in Appendix 1.

The polymodal length-frequency distributions of sea lamprey compiled for each stream were serially decomposed into their constituent modal size (age) classes using a computerized mathematical optimization procedure described by Macdonald and Pitcher (1979). The length data were grouped into 3 mm classes to smooth out artifacts in the data such as digit preferences. Components were assigned ages based on the number of years between lampricide treatments. The dates of lampricide treatments preceeding 1989 were as follows: Sturgeon River - May 31, 1985; Salem Creek - September 18-19, 1985; Bowmanville Creek - May 1-2, 1986; Cobourg Creek - May 7-8, 1986; Wilmot Creek - May 9-10, 1986; and Bronte Creek - May 12-14, 1986.

The six sea lamprey populations sampled in this study are continuously monitored and regularly treated with lampricide and therefore have well established length-frequency distributions (Holmes 1990). Each histogram was analyzed three times to ensure that the resulting parameter estimates (mean size, standard deviation, and proportions) were unique and were not dependent on starting values. No constraints, such as a constant coefficient of variation, were imposed when the final parameter estimates were obtained. Solutions were accepted when χ^2 goodness-of-fit values were small and standard errors were low relative to their respective parameter estimates. The final estimates were then projected onto the histogram and visually assessed for biological plausibility.

Macdonald and Pitcher (1979) recommend a minimum sample size of 50 individuals per age-group provided the researcher knows the number of age-groups in the population independently of the size-frequency analysis, as is the case in this study. Length data from my collections of sea lamprey were pooled with collections by control personel to ensure that a sample of approximately 200 or more was available for length-frequency analysis. The best results are achieved when the separation between modal sizes of consecutive age-classes is 2.5 to 3 times the component standard deviations (Grant et al. 1987; Grant 1989). Performance of length-frequency analysis deteriorates when either the sample size or separation criterion is not met, producing wide confidence intervals around the resulting parameter estimates. Therefore, the standard errors of the parameter estimates should be reported so that performance can be judged, particularly if constraints are imposed in the estimation procedure.

Results

Statolith Aging

Statoliths removed from sea lamprey larvae in Bowmanville and Salem Creeks deteriorated during storage. Few of the statoliths in the first subsamples from these populations were sufficiently intact to identify bands so a second set was prepared from each population. More than half of the statoliths removed from larvae collected in Bronte Creek also deteriorated, but it was logistically impossible to prepare a second subsample. Annuli were visible on 30 statoliths in the second subsample from Bowmanville Creek, but only two counts were possible as the statoliths deteriorated completely prior to the third count. Most of the deteriorating statoliths were transformed from hard structures into an amorphous gelatinous mass that disintegrated when attempts were made to position them on slides for better viewing. Because of the small subsamples of aged larvae from Bowmanville and Bronte Creeks the resulting estimates of size must necessarily be considered tentative.

Age could be assigned from statolith band number in nearly all larvae sampled from the Sturgeon River and Wilmot and Cobourg Creek collections and the second subsample of larvae from Salem Creek (Table 2). The frequency of ambiguous statoliths, that is, statoliths from which age could not be confidently assigned because all three annulus counts differed, varied from 0 to 0.1 for these populations (Table 2). Aged larvae from Bowmanville Creek had the highest frequency of ambiguous statoliths of all populations examined.

Table 2. Summary of the number (N) and mean lengths determined by statolith aging of six sea lamprey populations regularly treated with lampricide.

	Age 0+		Age 1+		Age 2+		Age 3+		Age 4+		Frequency of ambiguous counts	Index of average error (%)
	N	Mean length (mm)	N	Mean length (mm)	N	Mean length (mm)	N	Mean length (mm)	N	Mean length (mm)		
Bowmanville Creek			1	42.0	18	75.2	1	90.0			0.33	5.56[a]
Bronte Creek			7	42.6	8	88.8	4	107.0	1	151	0.048	7.85
Sturgeon River	2	18.5	22	54.9	20	80.0	3	125.6			0.06	15.7
Salem Creek	2	21.0	10	50.8	22	87.8	10	108.9	1	124	0.1	13.8
Wilmot Creek	4	21.2	17	57.2	16	86.6	13	116.8			0.0	8.86
Cobourg Creek			9	62.8	9	94.6	19	111.0	8	124.5	0.0	7.54

[a] Based on two counts rather than three as for the other populations.

Zero to three annuli were recognizable on statoliths from sea lamprey in the Sturgeon River and Salem and Wilmot Creeks and one to three annuli were counted on statoliths from larvae in Bronte and Bowmanville Creeks (Table 2). The age-composition estimated from annuli on statoliths was generally consistent with expectations based on treatment dates prior to 1989 in these tributaries, with the exception of the Sturgeon River where larvae with four annuli were also expected. The largest aged larvae from the Sturgeon River was 133 mm. Six larvae between 133 and 170 mm long were also collected for aging, but were inadvertently missed during subsampling. Larvae with four annuli collected from Bronte and Salem Creeks may have been residuals from the 1986 treatments. There was less than a 5% difference between the mean and median lengths of age-groups, therefore, mean lengths are shown in Table 2 for consistency with the length-frequency length estimates.

Statoliths with zero to three identifiable annuli were also anticipated in sea lamprey sampled from Cobourg Creek in 1989. However, young-of-the-year were not included in the subsample for aging, although several were collected during the lampricide treatment. Sea lamprey with four annuli (Table 2) may be residuals that survived the previous treatment in 1986.

The index of precision of aged sea lamprey larvae ranged from 5.6 to 15.7% (Table 2). These figures mean that repeated aging of a single larvae would result in the assignment of the same age to that larvae 84.3 to 94.4% of the time, depending on the population. Most of the error was associated with the determining the number of annuli in the oldest age-groups (2+, 3+). Bowmanville Creek had the lowest average error, but this may be an artifact since only two counts were made.

Length-frequency Analysis

Three age-groups were recognizable from the length-frequency distributions of sea lamprey larvae collected from Bowmanville, Bronte, and Cobourg Creeks and four age-groups were observed in the distributions compiled from the remaining populations treated in the summer of 1989 (Figure 2; Table 3). The age composition of populations from Cobourg Creek and the Sturgeon River were underestimated relative to expectations based on treatment history. These are the two populations in which statolith aging also underestimated age-composition (see above). The number of large individuals (> 150 mm) in both samples is too small for analysis.

The standard errors of the age-group parameter estimates (Table 3) indicate that the performance of the length-frequency analysis protocol followed in this study is reasonable. Performance was poorest with respect to data from Sturgeon River and Wilmot Creek in that the age-group standard deviations had the highest standard errors relative to their estimates. When either fewer or more components than shown in Table 3 were fitted, comparable χ^2 values could be obtained. However, with fewer components, the last component usually overlapped at least one adjacent component completely and occasionally partially overlapped a second component. In contrast, with more components the standard errors of the parameter estimates for at least one and sometimes two components, became unacceptably large, being

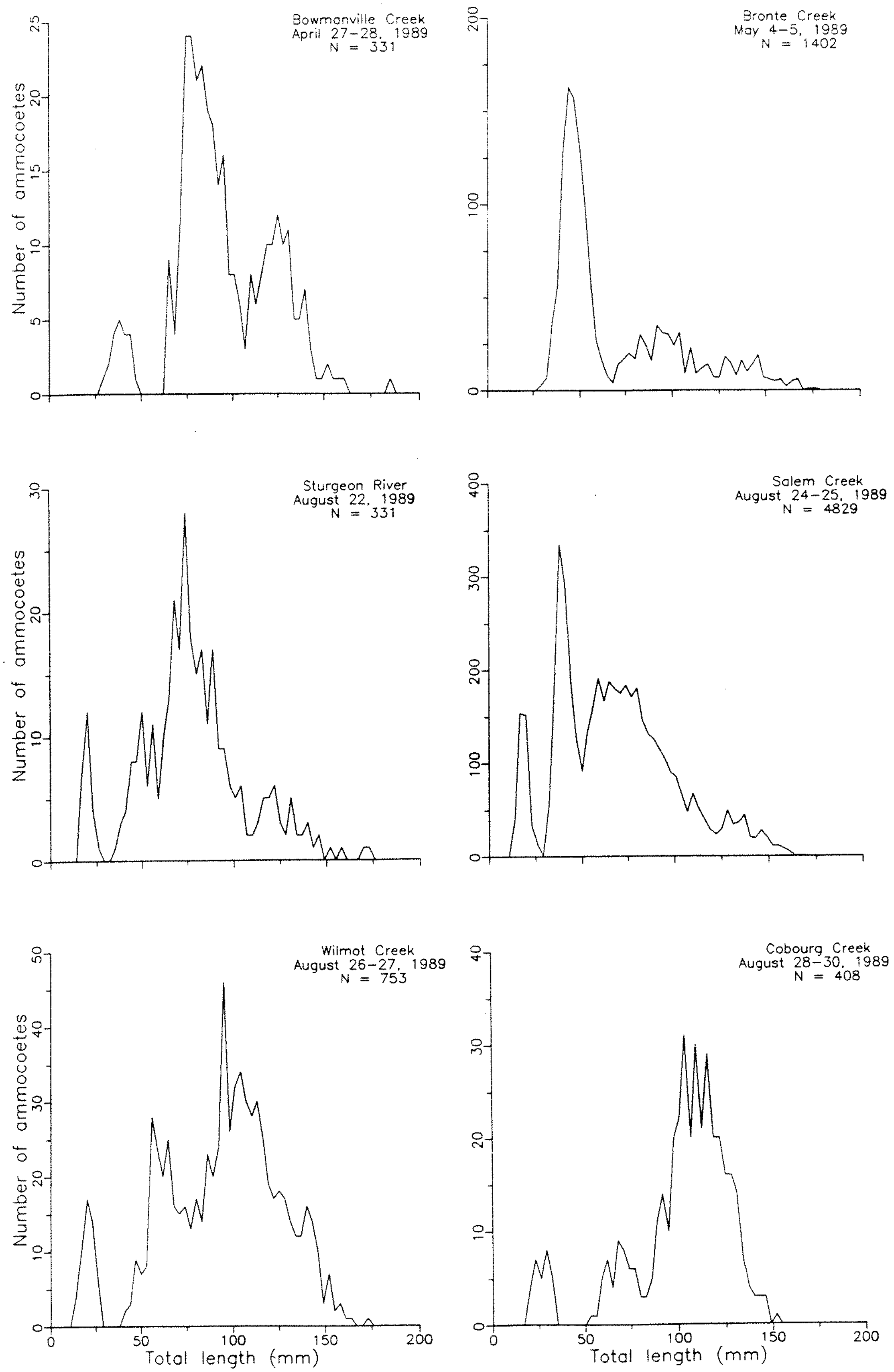

Figure 2. Length-frequency distributions of larval sea lamprey collected from six southern Ontario streams during lampricide treatments in 1989. Metamorphosing sea lampreys were collected during the August treatments, but are not shown here. Note the difference in scales used on the y-axis.

Table 3. Summary of mean lengths and standard deviations (SD) of the means from the best fits to the length-frequency distributions of six larval sea lamprey populations shown in Figure 2. Data were analyzed using Macdonald and Pitcher's (1979) methodolgy. Standard errors of the parameter estimates are shown in brackets.

Stream	Parameter	Age-group			
		0+	1+	2+	3+
Bowmanville Creek	Mean		38.3 (1.20)	81.8 (0.95)	122.8 (2.33)
	SD		4.9 (1.10)	9.4 (0.75)	15.0 (1.69)
Bronte Creek	Mean		46.0 (0.22)	91.4 (1.30)	138.6 (2.60)
	SD		6.22 (0.17)	15.0 (1.40)	14.7 (1.60)
Sturgeon River	Mean	19.1 (0.51)	46.6 (2.00)	75.6 (1.40)	120.6 (6.50)
	SD	2.13 (0.56)	5.88 (1.50)	12.4 (1.80)	17.6 (1.60)
Salem Creek	Mean	17.8 (0.13)	38.7 (0.16)	71.4 (0.58)	127.7 (1.90)
	SD	2.18 (0.12)	3.36 (0.16)	19.2 (0.59)	15.9 (1.10)
Wilmot Creek	Mean	19.9 (0.49)	60.2 (1.20)	101.0 (1.80)	137.6 (4.30)
	SD	3.28 (0.40)	8.19 (0.87)	15.8 (2.40)	11.1 (2.00)
Cobourg Creek	Mean	25.6 (0.79)	68.3 (1.40)	113.6 (0.89)	
	SD	4.1 (0.64)	7.2 (1.00)	14.5 (0.71)	

either the same magnitude or greater than the parameter estimate. Either case was rejected as unrealistic because it impied that the data do not support such an interpretation.

Comparison of Length Estimates

The estimates of sea lamprey mean lengths produced by analysis of the length-frequency distributions and annuli on statoliths are relatively similar at lengths of less than 90 mm (Figure 3). The largest deviations from the line of equality occurred at lengths greater than 90 mm due to lower estimates from statoliths. The number of large individuals in the statolith subsamples was small and it was generally more difficult to clearly assign ages using these statoliths. Neither method produced estimates which were consistently higher or lower than the other. Differences averaged 9.9 mm ± 8.6 (SD).

Discussion

Age determination of individual larval sea lamprey from statolith banding appears to validate the results of length-frequency analysis. There was good concordance in terms of the age-composition estimated by each method and that expected based on lampricide treatment history, and there was good agreement between estimated mean lengths less than about 90 mm. The length of larger (older) larvae was overestimated by length-frequency analysis relative to lengths estimated from statolith aging. Further aging of larvae > 90 mm is needed to confirm this assessment. Some other differences such as the recognition of residual 4+ yr old larvae (4 annuli on their statoliths) in Bronte, Salem, and Cobourg Creek were also

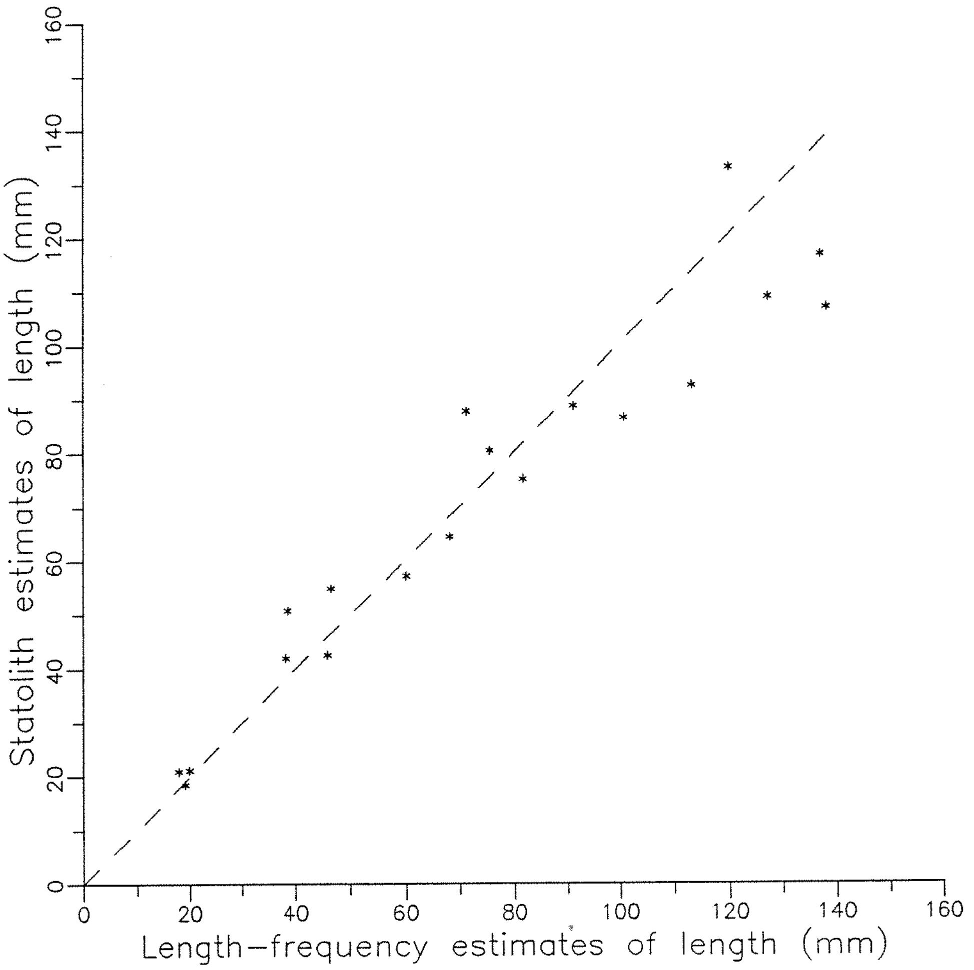

Figure 3. Comparison of estimated mean lengths obtained from length-frequency distributions and statolith aging. Dashed line is the line of equality. Each point represents the mean length estimates by both methods for one age-group from one population.

evident. Statoliths identified larvae that were 3+ yr old from Cobourg Creek, in contrast to the length-frequency analysis.

Neither method identified larvae that were 4+ yr old in the Sturgeon River as expected based on the treatment history of this population. The length-frequency findings suggest that either spawning did not occur or was not successful in 1985 or that the length distribution of the 4+ age-group completely overlapped adjacent age-classes. The statolith findings strongly support the former interpretation.

Age determination from statoliths indicates that age and mean length estimates derived by length-frequency analysis are reasonably accurate. This assessment applies only to larval sea lamprey subjected to regular and repeated lampricide treatments; it has less utility in untreated populations because density-dependent factors probably dominate the growth process (Holmes 1990), causing substantial overlap among older age-groups.

The success of size-frequency analysis at estimating age-composition of larval sea lamprey populations depends on sample size and the extent of overlap between different age-groups. Sample size varies with the number of age groups known or presumed present in the sample. The samples employed in this study were well above the suggested value of 200. The requirement that separations between mean sizes of consecutive age-groups be 2.5 to 3 times the component standard deviations for best results (Grant et al. 1987; Grant 1989) is also easily met in all the size distributions that were analyzed (Table 4). The decomposition

Table 4. Summary of sea lamprey sample sizes and separation of age-groups. Separation is expressed as the difference between adjacent means divided by the mean of the group standard deviations as shown in Table 3.

		Separation between age-groups		
Tributary	N	0-1	1-2	2-3
Bowmanville Creek	331		6.0	3.4
Bronte Creek	1402		4.2	3.2
Sturgeon River	331	6.8	3.2	3.0
Salem Creek	4829	7.5	2.9	3.2
Wilmot Creek	753	7.0	3.4	2.7
Cobourg Creek	408	7.6	4.2	

of length-frequency distributions into component age-groups is less likely to be successful if these criteria are not met.

My results do not imply a preference for length-frequency analysis now that statolith aging is becoming more readily achievable. The later approach to aging is superior in that a single estimate of age is provided from statolith annuli. Overlapping of length distributions generally increases with age, thus it is usually not possible to confidently age an ammocoete whose length is substantially different from the mean size of an age-group (Beamish and Medland 1988). If sample sizes are less than about 200 (assuming four age-groups), then good estimates will be difficult to obtain from length-frequency analysis without imposing constraints upon the estimation procedure, which require detailed justification, such as a constant coefficient of variation for each cohort or growth according to a von Bertalanffy curve (Grant et al. 1987; Grant 1989; France et al. 1991). In contrast, relatively small samples (approximately 50) are sufficient for estimating age-composition from statolith annuli and in fact larger samples become logistically difficult to process. The issue of sample size is an important consideration now as collection effort by the Canadian treatment units has been reduced in recent years.

An important assumption of this analysis is that age validation in other species or populations of lampreys applies to the populations observed in this study. Statoliths probably provide an accurate estimate of age over the range of more rapid growth since estimated ages were not greater than the maximum validated age of 5+. However, validation is the only way to determine that fish are not older than estimated and that older fish are not an important component of the population (Beamish and McFarlane 1983) and therefore should be applied to all populations. The aging results for the Sturgeon River and Cobourg Creek populations support this contention. The insistence on validation of all ages of larval sea lamprey in all populations studied may seem overly rigorous, but it is the only means by which an accurate understanding of population dynamics and life-history parameters can be assured.

Precision is a measure of the reproducibility of a measurement. The index of average error calculated in this study was higher (i.e., precision was lower) than reported for larvae of mountain brook lamprey (2.4%), sea lamprey (2.8-5.2%), and American brook lamprey (1.4-2.7%) in other studies (Medland and Beamish 1987; Beamish and Medland 1988). However, the frequency of ambiguous statoliths (0-0.33) was quite similar to the range of 0 to 0.27 reported by Volk (1986) for smaller samples of 5-30 sea lamprey from the upper Great Lakes. The index of average error and the frequency of ambiguous statoliths together provide a measure of the performance of the reader and should be reported in statolith aging studies. The lower precision in this study is probably related to the experience of the reader and problems with the preparation of statoliths from some populations.

The deterioration of statoliths experienced in this study was an unexpected difficulty with the preparation and storage protocol. The problem does not appear to be related to the storage medium, glycerol, since statoliths removed from larvae collected in the Sturgeon River and Wilmot and Cobourg Creeks did not deteriorate during storage. The storage period for these statoliths ranged from 130 to 153 days compared to 172 to 179 days for statoliths that deteriorated. These storage times are much greater than those used by other researchers (15-30 days; Medland and Beamish 1987, 1991; Beamish and Medland 1988) and may imply an upper limit of 150-160 days for storage in glycerol. The problem with the second subsample from Bowmanville Creek larvae, which were stored for 33 days in glycerol, suggests stream(population)-specific factor(s), for example, differences in calcium metabolism and reserves, may be an important consideration when using statoliths for aging. Storage times of 15-30 days, as used by other researchers, are acceptable for glycerol, judging from the second subsample from Salem Creek, but in this study such times were logistically impossible to regularly achieve.

Age determination of sea lamprey from statolith annuli is a labour-intensive procedure. Removing statoliths from the larvae, mounting them on slides, and counting the annuli three times required approximately 1.25 hours per pair of statoliths in this study. This does not include preparation and storage times in glycerol which varied from 130 to 179 days but could be shortened to 15-30 days in future studies. Thus the investment in terms of time that must be committed to statolith aging is significantly greater than the time required to produce similar information by length-frequency analysis.

The finding that statoliths and length-frequency analysis produce comparable estimates of age-composition is important for two reasons. First, length-frequency distributions are the only way of retrospectively examining the population dynamics of larval sea lamprey since the intiation of the control program. The validation of this technique provides some confidence in the accuracy of life-history parameters estimated from these data. Second, the use of statoliths in the control program is likely to increase in the future, particularly if collection effort during lampricide treatments remains at reduced levels. Statoliths provide the only means of determing life history parameters from small samples.

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Appendix 1. Identification codes, total length (mm), and annuli counts of statoliths subsampled from six larval sea lamprey populations in this study. A dash indicates no count was made.

ID Code	Total length	First count	Second count	Third count
Bowmanville Creek - April 27, 1989				
P6B7	80	2	2	2
P8A1	79	2	2	-
P8A2	89	2	2	-
P8A3	90	3	3	-
P8A4	85	2	3	-
P8A5	93	-	-	-
P8A6	84	2	2	-
P8A7	79	-	-	-
P8A8	90	-	-	-
P8A9	70	2	2	-
P8A10	84	2	2	-
P8A11	90	-	-	-
P8A12	75	2	2	-
P8B1	82	2	2	-
P8B2	75	2	2	-
P8B3	63	2	2	2
P8B4	73	2	2	-
P8B5	81	1	1	-
P8B6	64	-	-	-
P8B7	70	2	2	-
P8B8	73	2	2	-
P8B9	68	1	1	-
P8B10	72	-	-	-
P8B11	42	1	1	-
P8B12	127	-	-	-
P8C1	141	-	-	-
P8C2	110	-	-	-
P8C3	122	-	-	-
P8C4	121	-	-	-
P8C5	125	-	-	-
P8C6	113	-	-	-
P8C7	113	-	-	-
P8C8	122	-	-	-

Appendix 1. Continued.

ID Code	Total length	First count	Second count	Third count
P8C9	80	-	-	-
P8C10	117	-	-	-
P8C11	83	2	2	-
P8C12	75	2	2	-
P8D1	67	2	2	-
P8D2	76	2	-	-
P8D3	68	-	-	-
P8D4	77	-	-	-
P8D5	68	2	1	-
P8D6	111	3	2	-
P8D7	119	-	-	-
P8D8	116	-	-	-
P8D9	117	-	-	-
P8D10	91	2	1	-
P8D11	117	-	-	-
P8D12	97	3	2	-
P8E1	116	3	2	-
P8E2	38	-	-	-
P8E3	33	-	-	-
P8E4	66	-	-	-
P8E5	38	-	-	-
P8E6	64	2	2	-
P8E7	69	2	2	-
P8E8	68	2	3	-
P8E9	133	-	-	-
P8E10	64	2	2	-
P8E11	70	-	-	-
		Bronte Creek - May 4, 1989		
P1A9	105	2	3	3
P1A10	94	3	3	3
P1A12	96	3	3	3
P1B4	151	4	4	4
P1B6	106	3	2	2
P1B9	101	2	2	2
P1B10	73	2	2	2

Appendix 1. Continued.

ID Code	Total length	First count	Second count	Third count
PIB11	40	1	2	1
P1B12	35	1	1	1
P1C1	88	2	2	2
P1D1	135	3	2	3
P1D2	74	2	1	2
P1D3	39	1	1	1
P1D4	50	1	1	1
P1D5	81	2	2	2
P1D6	45	1	1	1
P1D7	43	1	1	1
P1D8	112	-	-	-
P7B8	96	-	-	-
P7B10	46	2	1	1
P7B11	50	1	-	-
P7B12	50	1	-	-
P7C1	100	1	-	-
P7C4	42	1	-	-
P7C5	43	-	-	-
P7C7	45	1	-	-
P7C9	40	1	-	-
P7C11	37	-	-	-
P7C12	49	1	-	-
P7D1	39	2	2	3
P7D2	42	-	-	-
P7D3	43	1	-	-
P7D5	48	-	-	-
P7D7	37	1	-	-
P7D8	33	1	-	-
P7D10	43	1	-	-
P7D12	48	1	-	-
P7E2	46	2	-	-
P7E3	75	1	-	-
P7E4	48	1	-	-
P7E5	74	-	-	-
P7E6	42	1	-	-
P7E7	51	1	-	-
P7E9	74	1	-	-

Appendix 1. Continued.

ID Code	Total length	First count	Second count	Third count
P7E10	97	3	-	-
P7E11	99	2	2	2
P7E12	88	2	2	2
Sturgeon River - August 22, 1989				
P1D10	102	3	3	3
P1D11	89	2	2	3
P1E2	81	2	2	2
P1E3	81	2	2	2
P1E4	81	2	2	2
P1E5	77	2	1	1
P1E6	79	2	2	1
P1E7	133	3	3	3
P1F2	63	1	1	1
P1F3	98	2	2	2
P1F4	79	2	2	1
P1F5	75	2	1	1
P1F6	66	1	1	1
P1F7	75	2	2	2
P1F8	80	2	2	2
P1F10	93	2	2	1
P1F11	82	2	2	2
P1F12	80	2	3	3
P1G2	87	2	1	2
P1G3	82	-	-	-
P1G4	73	1	1	1
P1G6	78	2	2	2
P1G7	66	1	1	1
P1G8	73	2	2	2
P1G9	142	3	3	2
P1F1	73	2	2	2
P1E1	91	2	2	2
P1F9	74	2	2	2
P1G11	74	2	2	2
P1H1	49	1	1	1
P1G5	81	2	2	2

Appendix 1. Continued.

ID Code	Total length	First count	Second count	Third count
P1H2	43	2	2	2
P1H3	45	1	1	1
P1H4	38	1	1	1
P1H5	63	0	0	1
P1H6	67	1	1	1
P1H8	71	-	-	-
P1H10	44	1	1	1
P1H11	53	-	-	-
P1H12	19	0	1	0
P2A1	18	0	0	0
P2A2	37	1	1	1
P2A3	45	1	1	1
P2A4	46	1	1	1
P2A5	54	1	1	1
P2A7	67	2	1	1
P2A8	53	1	1	1
P2A9	52	1	1	1
P2A10	54	1	1	1
P2A11	48	1	1	2
P2A12	41	1	1	0
P2B1	51	2	2	1
P2B2	49	1	0	0

Salem Creek - August 24, 1989

ID Code	Total length	First count	Second count	Third count
P6D10	20	0	0	0
P8F1	107	2	2	2
P8F2	107	3	3	3
P8F3	74	2	2	3
P8F4	73	2	3	2
P8F5	77	2	2	3
P8F6	95	3	2	2
P8F7	89	3	3	3
P8F8	76	2	3	3
P8F9	76	1	1	1
P8F10	70	2	2	2
P8F11	58	1	1	2

Appendix 1. Continued.

ID Code	Total length	First count	Second count	Third count
P8F12	65	1	0	1
P8G1	60	2	2	2
P8G2	58	2	3	3
P8G3	45	1	1	1
P8G4	47	1	1	2
P8G5	40	1	1	1
P8G6	45	1	1	1
P8G7	39	1	1	1
P8G8	42	1	1	1
P8G9	22	0	1	0
P8G10	20	-	-	-
P8G11	135	3	3	3
P8G12	134	3	3	3
P8H1	77	2	2	2
P8H2	109	2	1	2
P8H3	90	3	3	3
P8H4	90	2	2	3
P8H5	82	1	1	1
P8H6	107	3	2	1
P8H7	86	2	2	1
P8H8	75	2	2	2
P8H9	82	1	2	2
P8H10	107	3	2	2
P8H11	102	2	2	2
P8H12	110	2	2	1
P9A1	102	3	3	3
P9A2	105	3	4	3
P9A3	124	3	4	4
P9A4	106	3	3	3
P9A5	123	3	3	3
P9A6	90	2	2	3
P9A7	86	2	2	2
P9A8	94	2	2	2
P9A9	88	2	1	2
P9A10	98	3	3	3
P9A11	87	2	2	2
P9A12	67	1	2	3

Appendix 1. Continued.

ID Code	Total length	First count	Second count	Third count
P9B1	77	2	2	2
P9B2	59	2	3	1
		Wilmot Creek - August 26, 1989		
P2B4	87	2	2	2
P2B5	93	2	2	2
P2B7	113	3	2	3
P2B8	132	3	3	3
P2B9	113	3	3	3
P2B12	99	2	2	2
P2C1	84	2	1	2
P2C3	87	2	2	2
P2C7	56	1	0	1
P2C9	53	1	1	1
P2D1	56	1	2	2
P2D2	83	2	2	2
P2D3	79	2	2	2
P2D10	97	2	2	3
P2E1	121	3	3	2
P2E2	111	3	3	3
P2E5	67	2	3	2
P2E6	113	3	2	2
P2E7	129	3	3	3
P2E8	134	3	2	2
P2E9	133	4	3	3
P2E11	109	3	4	3
P2E12	104	3	3	3
P2F10	106	3	3	3
P2G1	102	3	3	3
P2G7	77	2	2	2
P2G11	75	2	2	2
P2H2	67	2	1	1
P2H3	62	1	1	1
P2H4	61	1	1	1
P2H5	60	1	1	1
P2H9	54	1	1	1
P2H10	41	1	1	1

Appendix 1. Continued.

ID Code	Total length	First count	Second count	Third count
P2H11	127	3	3	2
P3A1	24	0	0	0
P3A4	59	1	1	1
P3A7	91	1	2	2
P3A9	19	0	0	0
P3A10	58	1	1	1
P3A12	119	3	3	2
P3B5	54	1	1	1
P3B9	62	1	1	1
P3B10	55	1	1	1
P3C2	39	1	1	1
P3C8	18	0	0	0
P3C10	24	0	0	0
P3D2	18	-	-	-
P3D4	61	1	0	1
P3D7	64	1	2	2
P3D8	69	1	1	1
P3D9	61	1	1	1
Cobourg Creek - August 29, 1989				
P3E2	149	4	4	4
P3E7	115	-	-	-
P3E11	124	3	3	3
P3E12	105	2	1	2
P3F2	105	2	3	2
P3F6	98	2	2	2
P3F9	70	2	1	1
P3G8	110	3	3	3
P3G9	103	-	-	-
P3H1	94	2	2	3
P3H2	93	2	3	3
P3H4	133	4	4	4
P3H8	116	3	3	3
P4A5	119	3	2	3
P4A8	97	3	3	3
P4A9	94	3	3	3

Appendix 1. Continued.

ID Code	Total length	First count	Second count	Third count
P4A11	78	2	2	2
P3F6	98	-	-	-
P4A6	126	4	4	3
P4A12	89	2	2	2
P4B3	61	1	1	1
P4B7	55	1	1	1
P4B9	125	-	-	-
P4B11	121	3	4	3
P4C1	134	4	3	3
P4C7	113	-	-	-
P4C9	100	-	-	-
P4C10	91	-	-	-
P4C11	121	3	3	3
P4D2	101	3	3	3
P4D5	105	3	3	3
P4D6	122	4	3	3
P4D8	122	-	-	-
P4D11	117	4	4	4
P4D12	123	4	3	3
P4E1	107	3	4	3
P4E2	112	3	3	3
P4D4	124	4	4	4
P4E4	123	3	3	3
P4E5	122	6	4	4
P4E7	98	-	-	-
P4E8	125	-	-	-
P4F8	119	-	-	-
P4F12	61	1	1	1
P4G5	68	1	1	1
P4G6	120	-	-	-
P4H4	98	3	3	3
P4H7	67	-	-	-
P5A3	121	-	-	-
P5A8	136	-	-	-
P3F1	89	3	3	3
P3F3	91	2	2	2
P4B4	64	1	1	1

Appendix 1. Continued.

ID Code	Total length	First count	Second count	Third count
P4B5	65	1	1	1
P4B6	61	1	1	1
P3E8	128	4	4	3
P4G4	61	1	0	1
P3E1	97	3	4	4
P3F10	77	2	2	2
P3G5	115	3	2	2