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[Article]

An Assessment of Age Determination Methods for Great Lakes Larval Sea Lampreys

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Abstract.—Estimating the age composition and recruitment of populations of invasive sea lampreys *Petromyzon marinus* necessitates the validation and improvement of age assessment methods that rely on statoliths and length-frequency data. Determining age based on length-frequency distributions is subjective because of heterogeneity in the growth rates of larval sea lampreys (ammocoetes) within and across streams and the resulting overlap in lengths between age-classes. Statolith-based age assessment methods have never been validated for more than 1 year. We established "known-age" ammocoete populations in two streams by introducing a single cohort of spawners above barriers and compared estimates of ammocoete age based on statoliths with the true ages. In five additional streams, we used microsatellite data from adults and ammocoetes to assign parents to ammocoetes produced the same year and compared the age determined by statolith-based interpretation with the age based on parentage assignment. We combined length-frequency data with age composition data from streams having known-age populations and evaluated likelihood-based statistical models used to estimate the age composition of the ammocoete population. Multiple independent age readings of statoliths from known-age sea lampreys indicated that the age assessment bias (average percent error) was 24.3–36.2%. Genotype-based ages differed from statolith-based ages in 36.1% of cases across all streams. Bias-corrected statolith ages, when combined with length-frequency data, substantially increased age assessment accuracy in a known-age population stream and increased precision in a randomly selected stream; their use, however, requires knowledge of the magnitude of the bias of statolith-based age assessment in study streams. Further effort is needed to quantify these biases and generalize to different types of streams before a reliable methodology for age assessment will be available for sea lamprey management.

Recruitment, growth, and mortality in fish populations can be assessed by monitoring the age composition of the populations. Fish exhibit considerable plasticity in growth that is heavily influenced by the environment. Without accurate methods for age determination, use of environmentally plastic traits such as body size to infer age will bias estimates of population productivity (Campana and Thorrold 2001). Estimates of recruitment inform decisions about sustainable harvest rates for exploited fish populations. In the case of pest species, recruitment estimates can direct control efforts where the goal is to remove individuals at rates greater than those at which individuals can be replaced. In this paper we evaluate age assessment methods in an important pest species, the Great Lakes sea lamprey *Petromyzon marinus*.

Accurate assessments of the age of larval sea lampreys (ammocoetes) could allow managers to more effectively apply control measures by more accurately predicting the timing of metamorphosis. Adult sea lampreys spawn once in streams and then die, after which eggs hatch and the larvae burrow into the stream bottom, where they remain for an average of 3–6 years before metamorphosing into parasites and migrating to the lake, living there for up to 1.5 years before spawning. Treble et al. (2008) found that knowledge of ammocoete age improved length-based predictions of metamorphosis probability for individual sea lampreys. Models that better predict metamorphosis will enable managers to schedule times of treating streams with lampricides to maximize the cost-effectiveness of a chemical control program (Christie and Goddard 2003). Further, greater accuracy in age determination would improve models of sea lamprey stock–recruitment relationships, which are important for determining the expected effectiveness of nonchemical control tactics, such as trapping adults (Dawson 2007).

Historically, sea lamprey ammocoetes have been assigned ages by visual assessment of length-frequency distributions (Hardisty and Potter 1971; Beamish and Medland 1988). However, this method is subjective. Lengths of age-1 and older ammocoetes can overlap considerably, introducing uncertainty into estimates of

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the age composition of the population (Potter 1980); this has discouraged other analyses that require age composition data (e.g., estimates of recruitment or age-specific rates of mortality). Growth rates of sea lamprey ammocoetes have been related to water temperature, stream productivity, and ammocoete density (Holmes 1990; Rodríguez-Muñoz et al. 2001). Therefore, growth rates are likely to vary among populations—spatially among sections of the same stream, and even over time within stream populations—which contributes to size overlap between age-classes (Hansen et al. 2003).

Statoliths, the structure in lampreys analogous to otoliths in teleost fishes, have been used as an alternative technique to estimate the age of ammocoetes (e.g., Volk 1986; Beamish and Medland 1988; Hollett 1998). Statoliths are the only calcified structure found in lampreys; when viewed under transmitted light, they exhibit dark and light zones that have been interpreted as annuli (Volk 1986). Single-year validation of methods of statolith preparation and evaluation has been completed via oxytetracycline marking of ammocoetes (Beamish and Medland 1988) and ammocoetes undergoing metamorphosis (Hollett 1998). To use statoliths as a method of assessing age of larval sea lampreys, one must be able to validate that the banding pattern can be repeatably visualized by a reader and that the pattern represents the true age of the ammocoete over multiple years in contrasting streams (Jones 2007). Moreover, estimated ages greater than the maximum validated age must not be considered accurate (Beamish and McFarlane 1983). The studies just cited measured the precision (the deviation of each age assignment from the mean age assigned by readers) but not the accuracy (deviation of age assignment from the true age). In those studies, to validate age determinations made with statoliths, the investigators compared statolith-based ages with length-frequency data and with the amount of time after the most recent lampricide treatment. However, the most rigorous age validation method is the release of marked fish of known age into the wild, because the absolute age of such fish when recaptured is known without error (Campana 2001).

Further studies that concurrently use age assessment techniques other than ammocoete size are needed to validate statoliths as a viable method of age determination (Volk 1986). To validate statolith-based age assessment methods, one could over time collect statoliths from single cohorts of "known-age" sea lampreys or from ammocoetes of verified age based on individual- or time-specific marks. Because adult sea lampreys spawn only once, spawners could be introduced above barriers in 1 year and the resulting known-age ammocoetes produced could be monitored for up to 6 years. Moreover, genetic information obtained from adults released into Great Lakes streams can be used to assign parentage to individual ammocoetes collected in subsequent years (Derosier et al. 2007), another way to determine birth year. To assess the reliability of the age determination techniques in which individual ages are estimated by statolith interpretation or the age composition of a population is estimated from length-frequency data, we compared these estimates with the ages of known-age sea lampreys and with the ages of ammocoetes determined with genetic markers.

Schnute and Fournier (1980) described a statistical method for estimating age composition of a fish population from length-frequency data in which a von Bertalanffy growth function and an increasing variance of length with increases in ammocoete age are assumed. Fournier (1983) amended the approach to include information on age composition from a sample of fish from which a calcified structure was removed. Age information from a subsample of the fish population can determine the number of age-classes present, thereby helping avoid errors (Fournier 1983). Francis and Campana (2004) developed what they termed a "mixture model," whereby age composition is estimated from length-frequencies and partial ages, using maximum likelihood. Accurate age data can also help reduce bias from intrapopulation variability when estimating parameters of growth models (Smith et al. 1997), and bias can be statistically removed by using age assessment error matrices (Richards et al. 1992; Francis and Campana 2004).

Neither statolith-based methods nor statistical analyses of length-frequency data have been used by managers to assess the age composition of sea lampreys in Great Lakes populations, and both approaches warrant further evaluation. Our objectives in this study were to (1) determine the accuracy of statolith-based age assessment of ammocoetes, (2) apply and evaluate a statistical method for assessing ammocoete age that combines length-frequency data with partial age composition data; and (3) use genetic and statolith data to further assess the accuracy of statolith-based age assessments.

Methods

Assessment of statolith-based age determination by using samples having a known age.—We established populations of sea lampreys of known age in two streams by introducing 25 male and 25 female spawners above barriers (Figure 1). Big Garlic River, a cold, low-alkalinity (mean alkalinity = 52 mg/L $CaCO_3$) Lake Superior tributary, received spawners in

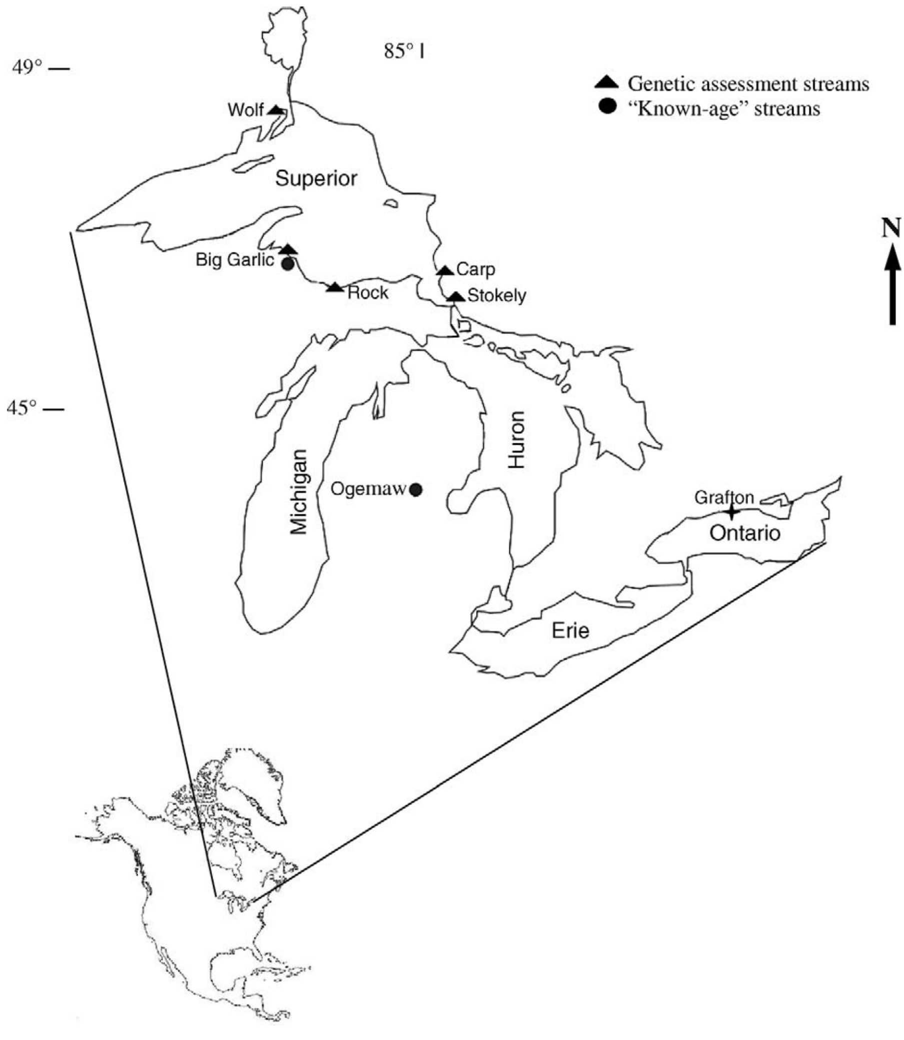

FIGURE 1.—Locations of streams in which sea lamprey populations were established to determine the accuracy of statolith-based age determination by using both sea lamprey populations of known ages and genetic assessment methods.

spring 2002; Ogemaw Creek, a warmer, high-alkalinity (mean alkalinity = 175 mg/L $CaCO_3$) Lake Huron tributary, received spawners in spring 2003. Spawners were introduced in 1 year above low-head barriers. In the year preceding introductions, virtually all ammocoetes from any previous stockings—which for both streams had occurred at least 3 years before the introduction of spawners for this study—were removed from both streams either through metamorphosis or treatment with a lampricide; therefore, no other age-classes of sea lampreys should have been present. Had any residual ammocoetes remained after treatment, they would have been large enough so as not to be confused with the known-age populations in this study. Each stream was sampled with an AbP-2 backpack electrofisher. The Big Garlic River was sampled in the summers of 2003, 2004, 2005, and 2006 when the ammocoetes would have had known ages of 1, 2, 3, and 4 years, respectively; Ogemaw Creek was sampled in 2004, 2005, and 2006 when the known ages of the ammocoetes were 1, 2, and 3 years, respectively.

Two sets of subsamples were used. One set was kept frozen until 2005 and one set was kept frozen until 2006; ammocoetes from each stream-year were randomly chosen for statolith evaluation. In an effort to prevent statolith readers from limiting their age determinations based on knowledge of the total number of age-classes, we added samples of statoliths from sea lampreys of unknown age collected from Bowmanville Creek ($N = 31$) and Silver Creek ($N = 12$), both Lake

Ontario tributaries, to the 2005 and 2006 data sets, respectively. Statoliths were prepared and evaluated based on a method that required transferring statoliths to individual wells within a multiwell plate filled with immersion oil, where they were stored for 9–18 d at room temperature to improve annuli visibility. As found in Volk (1986), viscous immersion oil facilitated maneuvering of the statolith to gain an optimum orientation for viewing the annuli. All statoliths were assigned a unique random number and linked back to the source sea lamprey after blinded assessments of age were conducted. Annuli were viewed and interpreted most clearly when the statolith was oriented laterally and viewed with a dissecting microscope (75×) and using transmitted light. However, we used a compound microscope (400×) and reflected light to substantiate our estimates of age. All statoliths were evaluated by two readers who read each statolith twice; statoliths for which interpretations were ambiguous were retained for further analyses.

Bias and precision were examined visually by age bias plots (Campana et al. 1995) and by comparing estimates of average percent error (APE). Age bias plots were constructed to compare the ages assigned by each reader with the true or mean age. The APE was calculated as follows:

$$100 \times \frac{1}{N} \sum_{j=1}^{N} \left(\frac{1}{R} \sum_{i=1}^{R} \frac{|X_{ij} - X_j|}{X_{ij}} \right),$$

where N is the number of statoliths read, R is the number of times each statolith was read, X_{ij} is the ith age determination of the jth statolith, and X_j is the reference age (Beamish and Fournier 1981). For bias assessment, the reference age was the true age. For precision assessment, the reference age was the average age determined by readers across all readings.

Applying a statistical model to estimate age distribution.—To combine length-frequency and statolith-based age information, we adapted the method described by Schnute and Fournier (1980) and Fournier (1983). The likelihood-based method we used for estimating age composition from length-frequencies and partial ages was previously developed and reported by Francis and Campana (2004), but with a reparameterization after Schnute and Fournier (1980). The method requires assumptions about how the fish in the population grow and how individual growth rates vary. These two assumptions allow prediction of the length-at-age composition of a mixed-age population, which can then be compared with actual data. Using maximum likelihood methods, we obtained parameters describing growth and variation in growth that best fit observed data and used these parameters to infer the age composition of a population. We assumed that mean length at age (μ_i) followed a von Bertalanffy growth function, as reparameterized after Schnute and Fournier (1980):

$$u_i = L_1 + (L_M - L_1)\frac{1 - k^{t-1}}{1 - k^{M-1}}; \quad i = 1, \ldots, M,$$

with t being the age-class, M being the maximum number of age-classes, L_1 being the mean length of the youngest age-class, L_M being the mean length of the oldest age-class, and k being the distance between two successive mean lengths (Schnute and Fournier 1980). We assumed that lengths for each age-class were normally distributed and that their standard deviation increased as a linear function of age. To estimate the most likely proportions at each age, we used a multinomial log-likelihood function implemented in AD model Builder (Otter Research 2000).

Partial age composition information was included by interpreting the statoliths from a subsample of the ammocoetes from the sample of those whose length was measured, corresponding to the "calibration" and "production" sample, respectively, described by Francis and Campana (2004). The observed proportion at age for a stream was determined by ages assessed with statoliths from a subsample of ammocoetes. The bias observed in age determinations made with statoliths from known-age streams was used to develop an age assessment error matrix and thus obtain the corrected proportion at age described by

$$p_a = \sum_{a'=1}^{A} C_{a,a'} \cdot p'_{a'},$$

where p and p' are the corrected and observed proportions at age, respectively; C is an age assessment error matrix, with rows a giving the true ages and columns a' the observed ages; and A is the total number of ages. We added a second multinomial likelihood term to the objective function that used this corrected proportion at age.

We tested the accuracy of the statistical model by estimating proportion at age for the two known-age populations when cohorts sampled from multiple years were combined into single length-frequency data sets. Using the statistical model, we then estimated the proportion at age by using only length-frequency information and compared the estimate with the "known" proportion at age. We also used these data to evaluate the statistical model when partial age composition data were included. The age assessment error matrices we developed to adjust bias in statolith-based age assessment were determined with statoliths from both streams with known-age populations. The statistical model estimated the most likely proportion at age, given both length and bias-corrected age information; we then compared the estimates with the known proportion at age.

We applied a similar method to estimate proportion at age by using length-frequency and statolith data from a stream known to have three age-classes of sea lampreys (Dawson 2007). Sea lampreys sampled in 2006 from Grafton Creek, a warm, high-alkalinity (mean alkalinity = 217 mg/L $CaCO_3$) tributary of Lake Ontario, were used to compare proportions at age estimated by the statistical model when only length information was used and those estimated by using both length and age information. Age information was obtained by taking a random sample of 31 statoliths from sea lampreys at least 25 mm in length and determining their age as described earlier. Ammocoetes 25 mm in length or less were estimated to be young of the year (M. T. Steeves, Department of Fisheries and Oceans Canada); statoliths could not be evaluated for these individuals because of their small size and thus they were not used in this analysis. To avoid potential bias of readers limiting their age interpretations to ages 1, 2, or 3, we added a sample of ammocoetes ($N = 5$) of unknown age, obtained from Ceville Creek, a Lake Michigan tributary, to the collection of Grafton Creek statoliths. Errors in age assessment were corrected in the model by using an age assessment error matrix based on statoliths from both streams with known-age populations. To evaluate the precision of the estimates of proportion at age for Grafton Creek, we derived approximate confidence limits from likelihood profiles.

Genetic assessment of accuracy of statolith-based age assessment.—In spring 1999, male and female adult sea lampreys were released in a 1:1 ratio (from 7 to 24 pairs of males and females) into the Big Garlic and Rock rivers located in the Upper Peninsula of Michigan, and the Carp River, Wolf River, and Stokely Creek located in Ontario, Canada (Figure 1). These streams are all cold, relatively low-alkalinity tributaries of Lake Superior with mean alkalinites of 52, 114, 18, 91, and 28 mg/L $CaCO_3$, respectively. These releases were a part of another study (Jones et al. 2003) in which low numbers of adults were stocked into these streams above low-head barriers that prevented adults not purposely stocked from entering the study areas. Before the sea lampreys were released in 1999, fin clips were collected from all adults for genetic analysis and stored individually in 95% ethanol.

During fall 2000, ammocoetes were collected by electrofishing from each of the five study streams. Adults had been introduced in years before 1999, so the ammocoetes we collected were not exclusively from the 1999 year-class. However, of the ammocoetes collected from each stream, 50 were within the typical size range for age-1 ammocoetes for each stream (Michael Twohey, U.S. Fish and Wildlife Service, Marquette, Michigan). The total lengths of all ammocoetes were recorded before their heads were removed for statolith removal and interpretation; their bodies were used for genetic analysis to establish parentage.

DNA was extracted and quantified from fin clips collected from the spawners released in 1999 and from ammocoetes collected the following year to establish parentage as described in Derosier et al. (2007). Microsatellite loci were amplified by using polymerase chain reaction (PCR) protocols developed for 10 loci (Bryan et al. 2003; Filcek et al. 2005). For the 10 loci, the mean number of alleles per locus, the mean expected heterozygosity, and the mean polymorphic information content are described in Bryan et al. (2003). The PCR products were obtained and scanned, and genotypes were assigned as described by Derosier et al. (2007). Each genotype was individually scored by at least two individuals, and any sample for which the scores disagreed was rerun.

Parentage analysis was performed in the program CERVUS (Marshall et al. 1998), which uses genotypes scored for Mendelian loci for both putative parents and offspring to determine the relative likelihoods of the parentage ascribed to parental-ammocoete genotype arrays. The most likely parental pair was assigned by using a two-step process as described in Marshall et al. (1998). Statistical confidence for each assignment was established on the basis of population allele and genotype frequencies at each locus, representing the likelihood that genotypes of the population of stocked adults in each stream matched genotypes of ammocoetes by chance alone (Marshall et al. 1998). Any offspring with genotypes inconsistent with those of all putative parental pairs in a stream were assigned to the age category of older than 1. Ammocoetes for which genotype matched at least one prospective pair of parents were assigned age 1. The latter assignment is less certain because parents with similar, but unknown, genotypes may have been released in previous years.

Statoliths were prepared and evaluated by using a method that required storing the statoliths in glycerin for 15 d to improve annuli visibility. Statoliths were then sealed in Crystal Bond adhesive and placed on microscope slides, which were assigned a unique random number; samples were linked back to a specific sea lamprey source after blinded age assessments were conducted. Statolith annuli were counted three times by one reader who used both a compound microscope and a dissecting microscope, as in Hollett (1998). When annuli counts for individual ammocoetes did not agree among the three assessments, those individuals were

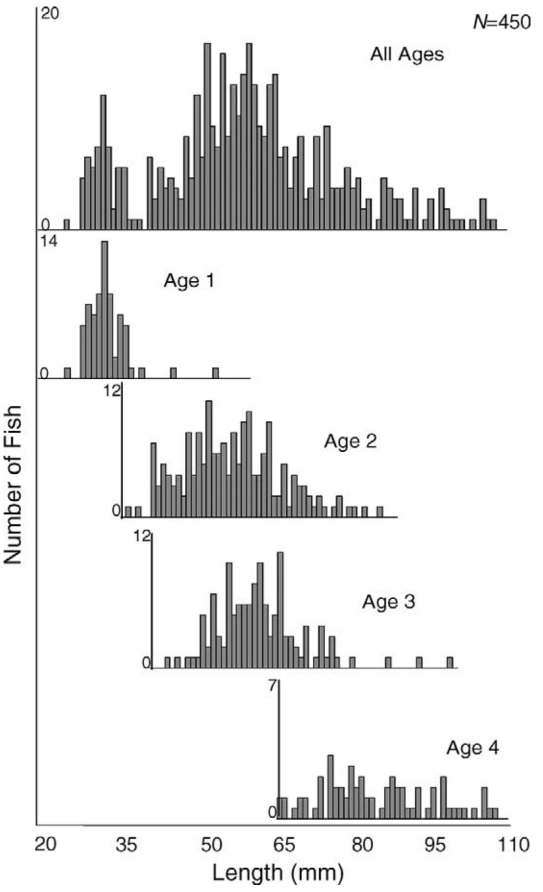

FIGURE 2.—Length-frequency distributions for all ages combined and for ages 1, 2, 3, and 4 individually for the Big Garlic River population of known-age sea lampreys after 4 years of sampling. Sea lamprey lengths ranged from about 20 to 110 mm; *N* indicates the total number of sea lampreys sampled.

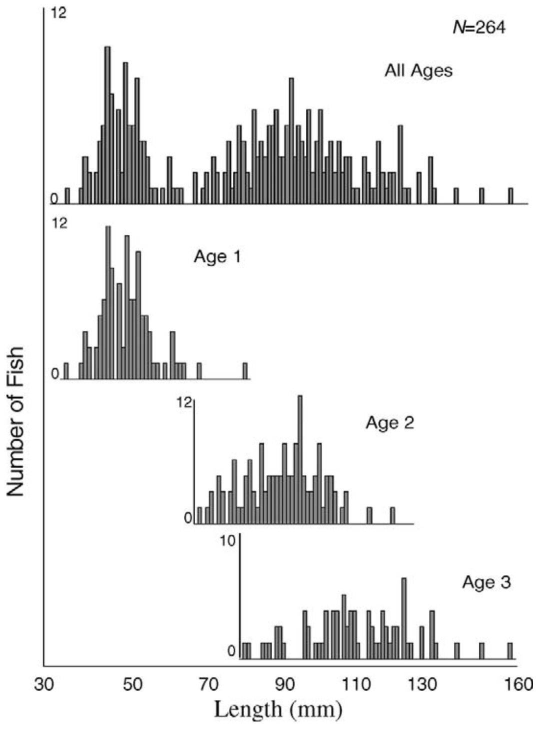

FIGURE 3.—Length-frequency distributions for all ages combined and for ages 1, 2, and 3 individually for the Ogemaw Creek population of known-age sea lampreys after 3 years of sampling. Sea lamprey lengths ranged from about 30 to 160 mm; *N* indicates the total number of sea lampreys sampled.

removed from the analysis. Statoliths were interpreted as having 1–4 annuli, the number of annuli being assumed to represent age; for the purpose of this study, individuals were assigned to either the age = 1 or age older than 1 category. Because of differences in preferences between statolith readers, this study used slightly different methods of preparing and evaluating statoliths than were used in the study of known-age ammocoetes. In a study by Dawson (2007), both methods of preparation and evaluation described here resulted in similar bias and precision estimates for assessing ammocoete age.

To assess accuracy, we compared statolith-based (phenotype) and genotype-based ages for each individual and assigned results to four categories: category 1, both methods assigned ammocoetes as age = 1; category 2, both methods assigned ammocoetes as age older than 1; category 3, phenotype age = 1 but genotype-based age older than 1; and category 4, genotype-based age = 1 but phenotype-based age older than 1.

Results

Assessing Statolith-Based Age Determination Using Samples of Known Ages

The length-frequency distribution of ammocoetes from the Big Garlic River and Ogemaw Creek for all years combined indicated effectively no modal separation among age-classes 2, 3, and 4 (Figure 2) and age-classes 2 and 3 (Figure 3), respectively. Sea lampreys from which statoliths were examined ($N = 141$) were a random sample from each population for each stream-year (Table 1). Overall, the bias APE was much larger (24.3–36.2%) than the precision APE (12.6–19.7%; Table 2), indicating a lack of accuracy in age assessments. The ages of sea lampreys from the Big Garlic River known to be age 1 were slightly overestimated, whereas those of ammocoetes age 2

TABLE 1.—Lengths of sea lampreys in the entire sampled population of "known-age" fish and those subsampled for statolith evaluation.

				Total length (mm)			
Location	True age (years)	Year collected	N[a]	Mean	SE	Minimum	Maximum
Entire sampled population							
Big Garlic River	1	2003	65	33	0.50	25	53
	2	2004	177	57	0.74	36	86
	3	2005	127	61	0.76	43	98
	4	2006	81	84	1.21	65	107
Ogemaw Creek	1	2004	92	50	0.76	34	81
	2	2005	99	90	1.00	69	119
	3	2006	73	112	1.80	80	155
Population subsampled for statolith evaluation							
Big Garlic River	1	2003	17	32	0.37	29	35
	2	2004	25	60	1.88	41	77
	3	2005	23	66	2.64	43	98
	4	2006	15	85	2.48	68	97
Ogemaw Creek	1	2004	23	49	1.27	39	57
	2	2005	23	87	1.55	75	98
	3	2006	15	106	4.72	80	155

[a] Number of sea lampreys or statoliths sampled.

and older were underestimated (Figure 4). Ages of Ogemaw Creek ammocoetes known to be age 1 or age 2 were overestimated, but that of age-3 ammocoetes was underestimated overall, being slightly overestimated by reader 1 and significantly underestimated by reader 2 (Figure 4). Within these populations among all readers, statolith interpretations tended to underestimate the variability in ages, and the apparent rate of annulus formation was less than 1.

Applying a Statistical Model to Estimate Age Distribution

The age assessment error matrices combined the data from both streams populated by known-age ammocoetes to adjust statolith-based proportions at age in the model for both known-age streams (Table 3). Estimated proportions at age based on the statistical models for the two streams having known-age populations when age composition data were included were very similar to the true proportions at age. The ability of the model to accurately estimate proportion at age was improved by adding bias-corrected age data to the Big Garlic River estimate (Table 4). However, model estimates of the proportion at age for Ogemaw Creek were very similar whether bias-corrected age and length were used or only length-frequency data were included. For Ogemaw Creek, age 1 was better estimated when only length was used, age 2 was better estimated by using length and bias-corrected age data, and the estimates of the proportion at age 3 were almost equal (Table 4).

Using only length information from the 2006 Grafton Creek population, our statistical model estimated over one-half of the population to be age 1, about one-fourth of the population to be age 2, and the rest age 3 (Table 5; Figure 5a). Statoliths ($N = 31$) were aged for a proportion of the population, and the model estimates that included bias-corrected age and length indicated that age-2 and age-3 ammocoetes made up 33% and 26% of the population, respectively, and that age-1 ammocoetes accounted for about 41% of the population (Table 5). With length and age information included, the model estimated that proportion at age consisted of fewer age-1 ammocoetes and more age-2 and age-3 ammocoetes when only length information was used (Table 5; Figure 5b). Adding the statolith information substantially increased the precision of the model's estimate of the proportion at age 1, as evidenced by a comparison of likelihood profiles for the estimate of proportion at age 1, with and without inclusion of age information (Figure 6).

TABLE 2.—Bias and precision of two readers using statoliths from sea lampreys of "known age" to determine age. Eighty fish were obtained from the Big Garlic River, 61 from Ogemaw Creek; APE is average percent error.

	Bias		Precision	
Reader	APE	SE	APE	SE
Big Garlic River				
1	28.3	4.06	12.6	1.66
2	24.3	2.47	13.4	1.69
Ogemaw Creek				
1	36.2	4.62	19.7	1.96
2	26.8	3.63	17.1	1.68

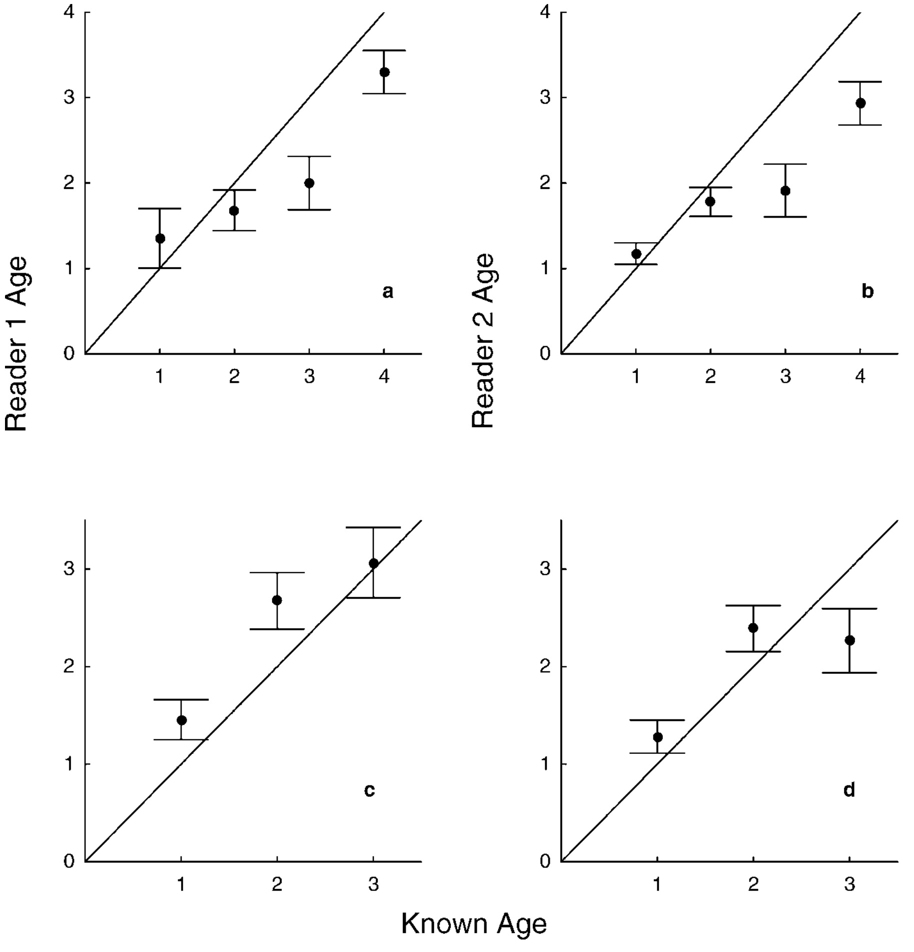

FIGURE 4.—Age bias plots for two known-age populations whose ages were determined from statoliths by two readers. Panels **(a)** and **(b)** compare the true ages with the average ages assigned by the readers to fish from the Big Garlic River population, panels **(c)** and **(d)** the true ages with the average ages assigned by the readers to fish from the Ogemaw Creek population. Error bars indicate 95% confidence intervals surrounding the average age assigned by each reader.

Genetic Assessment of the Accuracy of Statolith-Based Age Assessment

We observed 63.9% agreement between genotype- and statolith-based methods of assessing age of ammocoetes (Figure 7). Twenty-eight of the 250 ammocoetes (11.2%) were removed from analysis because the three statolith readings for each ammocoete were not consistent. Offspring were assigned to the age older than 1 category if genotypes were inconsistent with those of all putative parental pairs in a stream. When the phenotypic age disagreed with the genetic assessment of age older than 1, as in category 3, we concluded that these sea lampreys were incorrectly aged by the statolith technique. Paternal exclusion probabilities among loci ranged from 0.030 to 0.124 in

TABLE 3.—Age assessment error matrices used to remove bias from the observed proportion at age determined by statolith age determination when three and four age-classes are present.

	Statolith age			
True age	1	2	3	4
Three age-classes present				
1	0.6531	0.1481	0	
2	0.2041	0.5	0.5	
3	0.1428	0.3519	0.5	
Four age-classes present				
1	0.6531	0.1481	0	0
2	0.2041	0.5	0.3056	0
3	0.1428	0.3519	0.3056	0.5
4	0	0	0.3888	0.5

TABLE 4.—True proportions at age and those estimated by the model for the two streams with known-age sea lampreys using only length data and using both bias-corrected age and length data.

Age-class	True	Estimated using length only	Estimated using length and bias-corrected age
		Big Garlic River	
1	0.145	0.129	0.160
2	0.393	0.420	0.430
3	0.282	0.031	0.286
4	0.180	0.420	0.124
		Ogemaw Creek	
1	0.349	0.328	0.300
2	0.375	0.356	0.381
3	0.276	0.316	0.319

loci with only two alleles, and as high as 0.364 with as many as seven alleles. All five streams in this study probably had ammocoete growth rates similar to those observed in other Lake Superior tributaries such as in the Big Garlic River population of known age, and ammocoetes placed in category 3 had similar length distributions as for the age-2 age-class in the known-age Big Garlic River population (Figures 2, 7). The statolith technique tended to underestimate ages of smaller ammocoetes in these streams. Because of the relatively low probability of parental exclusion, especially in streams with a higher number of adults stocked, some individuals that were not offspring of adults we had stocked were assigned parents and placed in a separate category, category 4 for which we could not verify the correct age.

Discussion

Most previous assessments of the age composition of Great Lakes sea lampreys have relied on subjective interpretations of length-frequency data, despite widespread acceptance of the fact that accurate determinations are nearly impossible for age-classes older than age 1. Statoliths have been proposed as a potential alternative source for estimating ages of sea lampreys, but this method has not received sufficient validation to justify use of statoliths in programs assessing ages of sea lampreys. In the present study we found evidence for substantial biases in statolith-based age determinations for sea lamprey ammocoetes; this suggests that without a method for correcting such biases, statoliths do not offer a practical alternative or complement to length-based methods.

Our results revealed average biases of almost 30% (measured as APE) when we compared reader interpretations with the ages of known-age sea lampreys. Considering all data combined, ages of younger (age-1) ammocoetes were overestimated,

TABLE 5.—Proportions at age for Grafton Creek sea lampreys determined using only length data, only bias-corrected age data, and both bias-corrected age and length data.

Age-class	Length only	Bias-corrected age only	Bias-corrected age and length
1	0.577	0.336	0.407
2	0.264	0.360	0.330
3	0.159	0.272	0.263

estimates of age-2 ammocoetes were close to the true age, and ages of older (age-3, age-4) ammocoetes were underestimated. At the population level, this will tend to reduce the variability in ages among individuals in a sample and will bias estimates of survival. The apparent rate of annulus formation was less than 1 among all streams and readers, which suggests that within these populations either annuli do not form consistently in the statoliths or something in their interpretation confounds the age assignment.

Indications from other studies suggest that in some cases annulus formation either does not occur reliably on sea lamprey statoliths on a yearly basis or cannot be visualized. As has been observed in the otoliths of some teleost fishes in tropical environments, annuli do not form on statoliths in species of lampreys and populations that experience even growth throughout the year (Beamish and Medland 1988). During the phase of metamorphosis in which sea lampreys do not feed, and during the first few months of the juvenile period, when low ambient temperatures inhibit feeding, no annulus forms on the statoliths (Beamish and Medland 1988). Also, Barker et al. (1997) found that sea lampreys experiencing different ambient calcium ion concentrations displayed diversity in their reliability for estimates of age compared with estimates based on length-frequency distributions; in some sea lampreys from streams of low alkalinities, statoliths were absent altogether.

Because of limitations imposed by the chemical composition and size of statoliths, interpreting annuli formation on statoliths can be more difficult than observing annuli on otoliths in teleost fishes. Lamprey statoliths are composed primarily of calcium phosphate in a noncrystalline state, soft material that cannot be reliably sectioned or polished as otoliths can be. Statoliths must be read three-dimensionally after clarification in glycerin or immersion oil, and techniques such as scorching the statoliths to enhance the banding patterns are variably employed (Volk 1986). Annuli are often interpreted with dissecting scopes, which use lower magnification, probably because of the difficulty of using a compound microscope with a

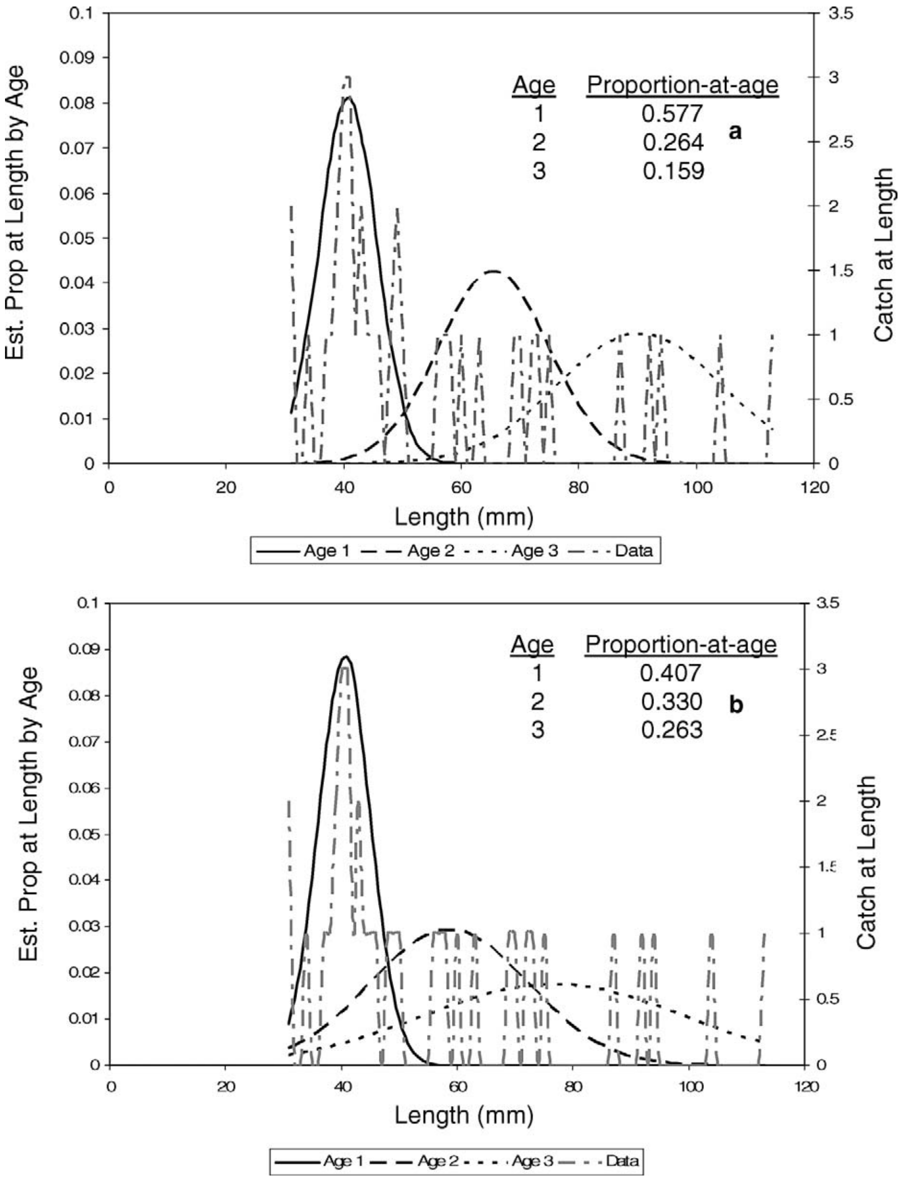

FIGURE 5.—Sample length-frequency data for the Grafton Creek sea lamprey population (gray lines) and the approximate age-class distributions produced by the model, indicated as the proportions of ages 1, 2, and 3. Panel **(a)** shows the age-class distributions approximated by the model based only on length data, panel **(b)** the approximations based on both length and bias-corrected age data.

single plane of focus to evaluate the three-dimensional structure of a statolith (Volk 1986; Beamish and Medland 1988; Hollett 1998). Lamprey statoliths are also much smaller than otoliths. Hollett (1998) found that in sea lampreys of an average total length of 148 mm, the average statolith length is 0.63 mm; in contrast, representative walleye pollock and arctic cod have fork lengths (otolith lengths) of 136 mm (6.5 mm) and 234 mm (5.7 mm), respectively (Short et al. 2006).

Our estimates of the precision of age assessment (measured as APE) for all age determinations were much higher than those reported for sea lampreys by Beamish and Medland (1988) and Hollett (1998): 2.8–

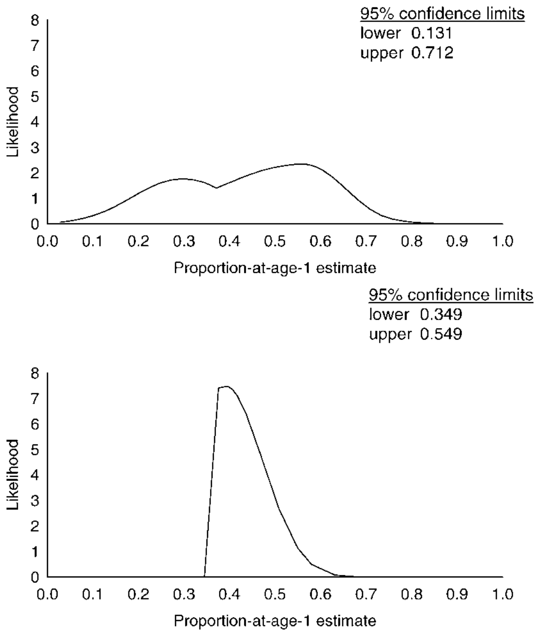

FIGURE 6.—Likelihood profiles for the model estimates of the proportion at age 1 for the Grafton Creek population of sea lampreys when using **(a)** only length data and **(b)** both length and bias-corrected age data.

5.2% and 0.8–2.4%, respectively. However, the exclusion of statoliths for which age could not be assigned or for which results were considered ambiguous (Beamish and Medland 1988; Hollett 1998) may have resulted in higher estimated precision in those studies. The average percent error in precision observed in this study was similar to that reported by Meeuwig and Bayer (2005) for Pacific lampreys *Lampetra tridentata* (16.7%) and western brook lampreys *L. richardsoni* (33%) in a study in which statoliths that were ambiguous or difficult to read were not excluded from analysis. Including in an analysis statoliths for which interpretations are ambiguous provides a more realistic assessment of the reliability of statoliths as a tool for age determination.

Our results provide some evidence that the biases in statolith-based age interpretations are related to growth rates of ammocoetes. In the warmer stream, Ogemaw Creek, where the ammocoetes experienced faster growth rates (Figures 3, 4), the age of ammocoetes was overestimated overall for ages 1 and 2 and underestimated for age 3, relative to their true ages. In the Big Garlic River, which is a colder stream with slower growth rates (Figures 2, 4), the age of ammocoetes was overestimated for age 1 but underestimated for age 2, age 3, and age 4, relative to their true ages. The genetic data suggested a failure of age assessment based on statoliths. Often, only one annulus was observed on statoliths of slow-growing ammocoetes, the true ages of which were older than age 1 (category 3, Figure 7). Because of uncertainty regarding the true age of ammocoetes that could be assigned to parents from the 1999 stocking (categories 1 and 4), the genetic data were less informative about the likelihood of age assessment using statoliths to overestimate the ages of fast-growing ammocoetes. Beamish and Medland (1988) also found that statolith growth patterns appear to be tightly correlated to ammocoete body size and growth rates.

We showed that accurate estimates of age composition could be obtained by combining length-frequency information with a small sample of bias-corrected statolith-based age composition data in a statistical model of the growth of sea lamprey ammocoetes. When only length-frequency information was used, the statistical model's proportion-at-age estimate for ages 3 and 4 for the known-age population in the Big Garlic River was far from accurate; the estimate was much improved when partial, bias-corrected age composition data were also included. For the Ogemaw Creek population the bias-corrected age composition data did not improve the proportion-at-age estimate for age 1, but did improve the estimate for age 2, and the estimate of age 3 was nearly equal to that obtained when only length data were used. Overall, when using length and bias-corrected age data, the model improved the proportion-at-age estimate for the Big Garlic ammocoetes and fared almost as well for the Ogemaw population as when only length data were used. Compared with using length alone to estimate proportion at age, adding bias-corrected age data to the model for the most part improved the proportion-at-age estimates for the older age-classes, where length-frequency data are unreliable (Jones 2007).

To test the utility of the statistical model, which is important for potential future applications by managers of sea lampreys, we estimated the proportion at age of ammocoetes from Grafton Creek, a randomly selected stream with an unknown proportion at age for this population. Model estimates of proportion at age for the Grafton Creek population revealed greater precision in the proportion-at-age estimate for age 1 when both bias-corrected age and length were used. The estimate based on using all available information was more precise, the 95% confidence interval with these data being much smaller than that obtained using length only (Figure 6). Addition of bias-corrected age composition data would also affect estimates of other demographic parameters such as age-1 abundance (or recruitment in some studies) and survival. By including a direct aging method independent of body size, we can

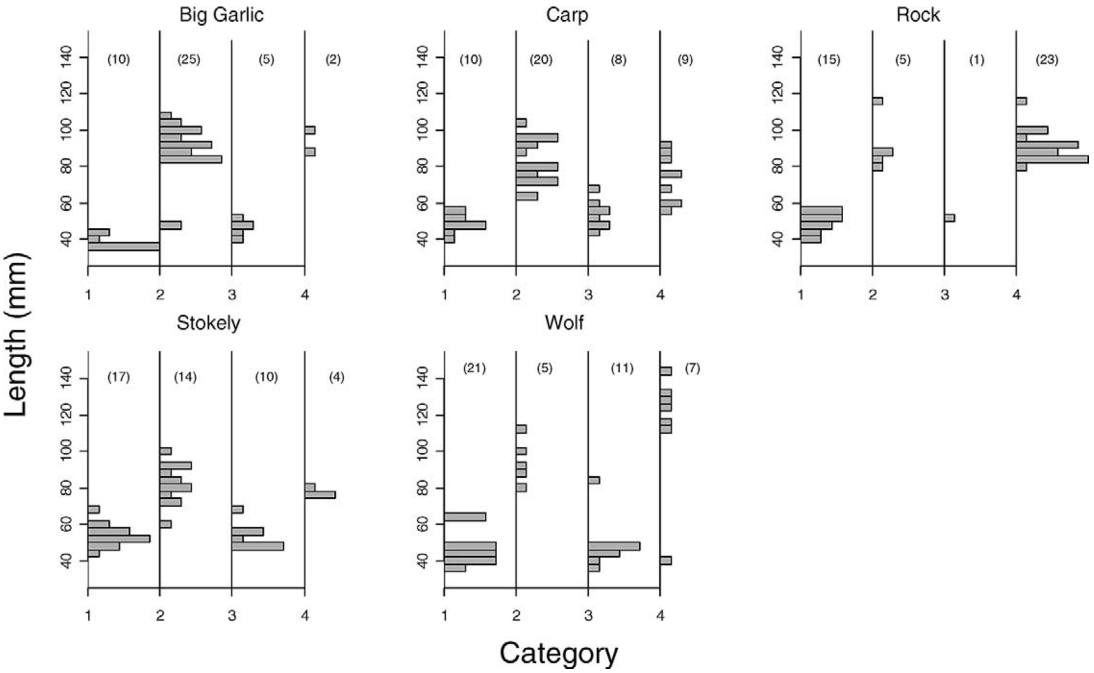

FIGURE 7.—Histograms of ammocoete length by category for each stream in the study used to assess the accuracy of statolith-based (phenotypic) age determination by genetic methods. The four categories are as follows: 1 = both methods determined ammocoetes to be age 1; 2 = both methods determined ammocoetes to be older than age 1; 3 = the phenotypic age was determined to be 1 but the genotype-based age to be greater than 1; and 4 = the genotype-based age was determined to be 1 but the phenotypic age to be greater than 1. The number of fish in each category is indicated in parentheses. The frequency of fish in each category on the *x*-axis ranges from 0 to 7.

analyze individual variability in the data, and our ability to investigate important life history parameters is greatly improved (Volk 1986). More precise age estimates will reduce the propagation of error through the assessment and management process (Morison et al. 2005).

At present, length-frequency analysis and statolith interpretation are the only known potential methods for assessing age of sea lampreys. Our results suggest that routine application of these methods to management of sea lampreys will require further research. A better understanding regarding the influence of environmental factors on the development of statoliths and the formation of bands typically associated with annual cycles of fast and slow growth is needed at this time. If this research can lead to models that predict the quantitative biases in statolith interpretations as a function of readily measured stream or environmental attributes, then statistical models can be effectively used to combine length-frequency and statolith information to obtain accurate estimates of age composition of sea lamprey populations.

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