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Ecology of recruitment in sea lamprey--summary

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Growth of larval sea lamprey from anadromous and landlocked populations

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Age discrimination and statolith diversity in sea lamprey from streams with varying alkalinity

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Gonadal variation in Great Lakes sea lamprey, *Petromyzon marinus*, larvae

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Potential fecundity of landlocked sea lamprey larvae, *Petromyzon marinus*, with typical and atypical gonads

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Unusual sex ratios in larval sea lamprey, *Petromyzon marinus*, from Great Lakes tributaries

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The diversity of statoliths and changes in statolith length were examined in larval and metamorphosing sea lampreys *Petromyzon marinus* from four streams in Ontario. In midsummer, increases in statolith lengths were similarly and positively correlated with larval total lengths in the four streams. Statoliths from larval and metamorphosing lampreys collected from Lynde and Farewell creeks in June and September displayed typical alternating opaque and translucent bands. The number of opaque bands, or annuli, provided reliable age estimates when compared with length-frequency distributions. In July and September, statoliths from some larval and metamorphosing lampreys collected from West Root River and Cannon Creek were either absent or did not have typical bands, hence they did not always provide reliable ages. The diversity of statoliths appears to be related to ambient calcium ion concentrations, especially during periods of rapid larval growth. The use of statoliths is sometimes the only method to age some populations of sea lampreys because of ambiguity in length-frequency distributions. The absence of statoliths, as encountered in this study, has potential management implications when determining age-at-metamorphosis.

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GREAT LAKES FISHERY COMMISSION

Project Completion Report

Age discrimination and statolith diversity in sea lamprey from streams with varying alkalinity

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Abstract.— The diversity of statoliths and changes in statolith length were examined in larval and metamorphosing sea lampreys Petromyzon marinus from four streams in Ontario. In mid-summer, increases in statolith lengths were similarly and positively correlated with larval total lengths in the four streams. Statoliths from larval and metamorphosing lampreys collected from Lynde and Farewell Creeks in June and September displayed typical alternating opaque and translucent bands. The number of opaque bands, or annuli, provided reliable age estimates when compared to length-frequency distributions. In July and September, statoliths from some larval and metamorphosing lampreys collected from West Root River and Cannon Creek were either absent or did not have typical bands, hence not always providing reliable ages. The diversity of statoliths appears to be related to ambient calcium ion concentrations, especially during periods of rapid larval growth. The use of statoliths is sometimes the only method to age some populations of sea lampreys due to ambiguity of length-frequency distributions. The absence of statoliths, as found in this study, has potential management implications when determining age-at-metamorphosis.

Age determination of larval sea lamprey has important management implications. Age-at-metamorphosis is used in the management of sea lampreys in the Great Lakes to determine how often streams are treated with 3-trifluoromethyl-4-nitrophenol (TFM). Historically, aging of larval sea lamprey has relied on the use of length-frequency distributions. This method requires large sample sizes and older age classes are often difficult to discriminate due to overlapping length distributions (Potter 1980).

Recently statoliths, analogous in form and function to the teleost otolith (Carlstrom 1963), were found to be useful to age lampreys where seasonal changes in growth are induced by environmental change (Volk 1986; Medland and Beamish 1987, 1991). In such cases, statoliths display alternating opaque and translucent bands similar to those in the teleost otolith (Studnicka 1912; Carlstrom 1963). The narrow opaque band represents prolonged slow growth occurring over the winter with cold temperatures and decreased feeding, while the translucent band represents rapid growth accompanying increased temperatures and feeding (Medland and Beamish 1987). When determining age using statoliths, each opaque band, or annulus is counted as representing one year. This count should accurately estimate age unless resorption has occurred, as documented for otoliths (Mugiya and Uchimura 1989) and scales (Bilton 1974; Liew 1974) in teleost fishes in response to environmental stress, including reproduction and starvation. The purpose of this study was to investigate the diversity of statoliths and their banding patterns in larval and metamorphosing sea lampreys in four streams of differing abiotic characteristics.

Methods

Larval and metamorphosing sea lampreys were captured by electrofishing. Larvae were collected from Lynde Creek (N = 839), and Farewell Creek (N = 1189) (latitude 43° 52' 32"N, longitude 78° 57' 43"W), tributaries to Lake Ontario on June 7 and June 14-16, 1995 and West Root River (N = 1,874) and Cannon Creek (N = 4012) (latitude 46° 33' 49"N, longitude 84° 16' 55"W), tributaries to the north shore of Lake Huron on July 20-24, 1995. Additional lamprey were collected from these streams September 15-23, 1995 (sample sizes: Lynde Creek, 374; Farewell Creek, 305; West Root River, 458; and Cannon Creek, 980). All the lampreys collected were used to generate length-frequency distribution for each stream at each sample time. A sub-sample of these lampreys were killed with an overdose of MS-222 (tricaine methanesulfonate) and immediately frozen for later removal of statoliths. During transport to the laboratory the lampreys were packed on ice to prevent thawing, and once at the laboratory, lamprey were frozen at -20°C.

Otic capsules were dissected from thawed lampreys and the largest of the statoliths, the sagitta was removed. Pigment was cleared by storing statoliths in 100% glycerin for 5-15 d. Age in years was equated to the number of opaque bands, or annuli, on the statolith following the criteria described by Volk (1986). Each statolith was labelled so that age estimation was not biased by prior knowledge of total length of the individual. Total length of statoliths was measured between the edges of the long axis of the statolith base using an ocular micrometer and a light microscope. Slopes and intercepts of the regressions relating total length of lamprey to statolith length were calculated and examined for statistical significance using

analysis of covariance (ANCOVA; Wilkinson 1990).

Results

Statolith Presence and Accuracy of Aging

Larval Lampreys.---All larval lampreys (N = 33; range, 68-165 mm) examined from Lynde Creek in June contained statoliths (range, 210-320 μm) that displayed characteristic alternating opaque and translucent bands. Ages assigned from the number of annuli were consistent with ages derived from the length-frequency distribution (Figure 1A). Larval lampreys (N = 11; range, 57-150 mm) examined from the September sample contained statoliths (50-220 μm) that also displayed typical alternating opaque and translucent bands. Ages assigned from the number of annuli were again consistent with ages estimated from the length-frequency distribution (Figure 1B). Larvae (N = 44; range, 37-154 mm) examined from Farewell Creek in June all contained statoliths (140-295 μm) with alternating opaque and translucent bands. Ages assigned from the number of annuli were again consistent with ages determined from the length-frequency distribution (Figure 1C).

Statoliths were found in only 13 of the 25 larvae examined from West Root River in July. For those larvae (N = 13; range, 46-133 mm) with statoliths (90-220 μm), the assignment of age from the number of annuli was not supported by the length-frequency distribution. For example, one larva of 40 mm total length estimated to be 1 year old from the length-frequency distribution, contained a relatively large statolith, 260 μm , bearing three annuli. Larvae (N = 5; range, 62-103 mm) examined from the September sample had statoliths (100-180 μm) displaying alternating opaque and translucent bands; however, ages

assigned from the number of annuli were again not supported by the length-frequency distribution. Larval lampreys (N = 18; range, 38-179 mm) examined from Cannon Creek in July all contained statoliths (80-300 μm) with typical banding patterns. Ages determined from the number of annuli were consistent with ages determined from the length-frequency distribution (Figure 1E).

Metamorphosing Lampreys.---Metamorphosing lampreys (N = 11; range, 129-154 mm) examined from Lynde Creek in September all contained statoliths (260-350 μm) with typical banding patterns and ages assigned were consistent with ages determined from the length-frequency (Figure 1B). All metamorphosing lampreys (N = 12; range, 142-161 mm) examined from Farewell Creek contained statoliths (325-440 μm) with typical banding patterns. Ages assigned from the number of annuli were consistent with ages estimated from the length-frequency distribution (Figure 1D).

Three metamorphosing lampreys (range, 155-161 mm) from West Root River were collected in July, and only two contained statoliths (both 210 μm). The assignment of age based on the number of annuli was not consistent with ages estimated from the length-frequency distribution. Metamorphosing lampreys examined in September seldom had statoliths. Statoliths were found in only 4 of 15 lamprey (155-169 mm) collected. These statoliths were small, 110-140 μm , and banding patterns were not visible. Metamorphosing lampreys (150-180 mm) examined from Cannon Creek in September (N = 9) did not contain statoliths. A χ^2 contingency test was used to determine if statolith presence was random between northern and southern streams. This test showed that there was an effect between northern and southern streams, hence statolith presence was not random ($\chi^2 = 59.37$, $P \leq$

0.005).

Regressions of Statolith Length on Total Length

Larval Lampreys.---The regressions of statolith length on larval length (S-L regression) for June collections in both Lynde and Farewell Creeks was significant (Table 1). The S-L regressions for July collections of larvae in both West Root River and Cannon Creek were significant, as well as the S-L regression for September collections of larvae in both West Root River and Lynde Creek (Table 1).

Metamorphosing Lampreys.---The S-L regression for the September collections of metamorphosing lampreys from Lynde Creek and West Root River was not significant (Table 1). However, the S-L regression was significant for metamorphosing lamprey examined from Farewell Creek in September (Table 1). Analysis of covariance for both larvae and metamorphosing lampreys in the four streams at both collection times, indicated that the slopes of the S-L regressions did not differ significantly ($P > 0.05$) except for three cases. For Lynde Creek, the S-L regressions for larvae collected in June and September were significantly different ($R^2 = 0.860$, $P \leq 0.05$), as were the S-L regressions for larvae collected in June and metamorphosing lampreys collected in September from Farewell Creek ($R^2 = 0.935$, $P \leq 0.05$). The S-L regression for larvae collected in June from Lynde Creek and metamorphosing lampreys collected from Farewell Creek were significantly different ($R^2 = 0.867$, $P \leq 0.05$).

Discussion

Statoliths in lampreys and scales and bones in teleosts are composed of calcium phosphate (Carlstrom 1963; Cameron 1990), which is acquired from the environment. In

teleosts, under circumstances of high demand for calcium phosphate, particularly when the ambient concentration is low, resorption from hard tissues may occur. For example, scale resorption has been reported in healthy fish during spawning (Fagade 1973) and for fish in poor nutritive condition (Regier 1962; Bilton 1974; Liew 1974). A change in statolith size, or in the extreme its absence from season to season (as observed in this study) is suggestive of such resorption. Statoliths were absent most often in lampreys undergoing metamorphosis, a period during which metabolic shifts occur for the physiological and morphological changes that take place during this period (O'Boyle and Beamish 1977).

Larval lampreys exhibiting the most diverse changes in statolith size between different life stages occurred in streams in which calcium ion concentrations were particularly low. Thus, in both Cannon Creek and West Root River, alkalinity was low ($< 30\text{mg/L}$ as CaCO_3), whereas in Lynde and Farewell Creeks, where larval lamprey statoliths exhibited no irregularities in size or presence, alkalinity was much higher (200 mg/L as CaCO_3). However, absence of statoliths in larval sea lamprey has also been observed in a high alkalinity stream, Shelter Valley Creek (M. Steeves, Department of Fisheries and Oceans, personal communication).

Statoliths from larval and metamorphosing sea lampreys displayed diversity in their reliability for estimating age. In southern, high alkalinity streams, the number of annuli agreed with the ages assigned from the length-frequency distribution. For example, ages from the number of annuli for larvae and metamorphosing lampreys examined in midsummer from Lynde and Farewell Creeks were consistent with ages derived from their respective length-frequency distributions. In contrast, in northern, low alkalinity streams where resorption of

statoliths may have occurred, the assignment of age was less reliable. Thus, whereas statoliths from larval lampreys collected in midsummer from West Root River exhibited banding patterns, the assigned ages were at variance with those determined from the length-frequency distribution. The inability to determine lamprey ages precisely by using statoliths has potential management implications because age cannot be discriminated from the length-frequency distribution in some populations because of excessive overlap. Sea lampreys in the St. Mary's River, a tributary to Lake Huron, cannot be assigned age from their length and statolith aging is the only method currently available (Steeves, personal communication).

In midsummer, the change in statolith size with total length was less than that in autumn, perhaps the result of small sample sizes for the autumn collections (for example, in Lynde Creek, the sample size in June was 33 versus 11 in September). The decrease in predicted statolith size for larvae of the same total length from June to September may be due to uncoupling of the linkage between somatic and statolith growth (Marshall and Parker 1982; Campana 1984). In some teleosts during periods of slow somatic growth, otoliths grow more rapidly and the converse is also true (Casselman 1990; Secor and Dean 1989). During winter, larval lamprey may undergo arrested or negative growth (Hardisty and Potter 1971), while statoliths continue to deposit material, hence the formation of opaque bands (Medland and Beamish 1987).

The results of this study indicate that sea lamprey statoliths are diverse in size and presence within and among populations over a single growing season. Also statoliths may not always provide a reliable estimate of age. This inconsistency emphasizes the importance of using length-frequency distributions in combination with statolith aging to verify assigned

ages.

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Legend for Figure 1

Length-frequency distributions smoothed by moving averages of seven (Hardisty and Potter 1971; Beamish and Medland 1988) for sea lamprey larvae from Lynde, Farewell and Cannon Creeks. For an example of age comparison from statolith aging and length-frequency distributions, ages determined from statolith banding for a sample of larvae is given above the length-frequency distribution. Shaded area represents metamorphosing lamprey.

TABLE 1.— Regression coefficients for larval (L) and metamorphosing (M) sea lampreys for the regression of statolith length on total length and predicted statolith lengths for the lamprey total lengths of 80, 110, and 140 mm for the regressions. All coefficients are significant ($P \leq 0.05$): N = number of fish; CL = confidence limits.

Stream and month	Life stage (N)	Intercept (CL)	Slope (CI)	Predicted statolith length (μm) at a lamprey length of :		
				80 mm	110 mm	140mm
Lynde Jun	L (33)	170.9 (141.0,201.0)	0.9 (0.6,1.1)	241.1	267.4	293.7
Sep	L (11)	1.8 (-65.5,69.1)	1.4 (0.8,2.0)	114.2	156.4	198.5
Farewell Jun	L (44)	133.0 (119.1,147.0)	1.1 (0.9,1.3)	221.0	254.0	287.0
Sep	M (12)	-198.7 (-539.7,-250.5)	3.8 (0.7,6.8)	a	a	331.4

Stream and month	Life stage (N)	Intercept (CL)	Slope (CI)	Predicted statolith length (μm) at a lamprey length of :		
				80 mm	110 mm	140mm
West Root Jul	L (12)	57.9 (-38.5,154.2)	1.3 (-1.5,4.0)	158.5	196.3	234.0
Sep	L (5)	-17.1 (-60.7,26.5)	1.9 (-3.5,7.4)	138.7	197.1	255.6
Cannon Jul	L (18)	107.6 (50.2,165.0)	0.7 (0.1,1.4)	165.3	187.0	208.7

^aStatolith sizes for metamorphosing lampreys were predicted for total length of 140 only.

