

Age, growth, and sex ratio among populations of least brook lamprey, *Lampetra aepyptera*, larvae: an argument for environmental sex determination

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Received 5.10.1992

Accepted 23.9.1993

Key words: Density-dependent sex determination, Adaptive significance, Sex-specific recruitment, Agnatha, Cyclostomata

Synopsis

Sex ratios of least brook lamprey, *Lampetra aepyptera*, larvae varied widely among 12 geographically-diverse streams of the eastern United States. The extremes were 29 and 71% male, and the proportion of males increased significantly with relative population density, which was estimated among the streams from the number of larvae collected per m² of substrate. The skewed sex ratios were not likely due to differential mortality between the sexes or differential recruitment to the adult stock, since they were established at the time of gonadal differentiation (at ca. 2 years of age) and remained relatively constant over the subsequent 2–3 years of larval life. Furthermore, although females seemed to predominate in the oldest larval age class, thus appearing to metamorphose later than males, their numbers were small and were omitted from the overall sex ratio. Sex ratio did not vary significantly with water hardness, pH, annual thermal units, or latitude. The possible adaptive significance of density-dependent sex determination in lampreys, however, remains elusive. It has been proposed that growth-promoting conditions might yield female-biased sex ratios as a tactic for ensuring that relatively large individuals become females, thereby increasing their fecundity. As predicted, larval size at a given age was generally greater in low-density populations, but there was no relationship between sex ratio and larval size, and female larvae were not consistently larger than the males.

Introduction

In some organisms, sex is determined by the environment that an individual encounters, rather than being fixed by genotype at conception (Charnov & Bull 1977, Conover 1984). Such environmental sex determination (ESD) has been known to occur in invertebrates such as parasitic nematodes (Christie 1929, Ellenby 1954), copepods (Christie 1929), and echiurid worms (Jaccarini et al. 1983), and skewed

sex ratios in some isopods (e.g., Legrand & Juchault 1972, Williams & Franks 1988) have led to similar speculations of ESD. Numerous experimental studies have also demonstrated that ESD is common among many poikilothermic vertebrates. Incubation temperature, for example, has conclusively been shown to influence sex determination in many turtles (e.g., Bull & Vogt 1979, Pieau & Dorizzi 1981, Gutzke & Paukstis 1984, Mohanty-Hejmadi & Diamond 1986), an alligator (Ferguson & Joanen 1982),

and a teleost fish (Conover & Kynard 1981). Population density, likewise, altered sex ratio in the European eel, *Anguilla anguilla* (D'Ancona 1950), and threespine stickleback, *Gasterosteus aculeatus* (Lindsey 1962), and low water pH produced a predominance of male progeny in 6 species of livebearing teleosts (Rubin 1985).

Sex differentiation in lampreys may also be sensitive to environmental influence, especially during their prolonged period of sexual indeterminacy (Okkelberg 1921, Hardisty 1965a, b). A small but variable excess of males has long been noted among spawning adult lampreys (e.g., Dean & Sumner 1898, Young & Cole 1900, Wigley 1959, Zanandrea 1961), and a positive relationship between the proportion of males and adult abundance has been observed (Hardisty 1954, 1961a). In particular, among the landlocked sea lamprey, *Petromyzon marinus*, of the upper Great Lakes, the sex ratio of both adults and larvae varied widely with abundance (Purvis 1979). As lamprey numbers were drastically

reduced following treatment of their natal streams with the lampricide 3-trifluoromethyl-4-nitrophenol (TFM), the proportion of males correspondingly declined and a predominance of female larvae and adults was soon observed (Smith 1971, Purvis 1979). Since the sex composition of transformed lampreys collected during the treatment was nearly identical to that of untreated lampreys migrating from upstream (Manion & Smith 1978), TFM appears not to be differentially toxic to males and females. It has therefore been suggested that sex differentiation in lampreys, at least the sea lamprey of the upper Great Lakes, is influenced by population density (Purvis 1979).

Consequently, the objective of the current study is to determine whether, despite life cycle and geographic differences, a similar relationship between sex ratio and population density exists in another species of lamprey, the least brook lamprey, *Lampetra aepyptera* (Abbott, 1860). This nonparasitic lamprey, which is distributed in small, cool

Table 1. Collection sites for least brook lampreys.

Stream	Date	River system	County and State	Latitude and longitude
Unicorn Branch	13 Apr 1987 15 Dec 1988 ¹	Chester R.	Queen Anne's Co., MD	39°13'–39°15'N 75°50'–75°51'E
Jordan Branch	16 Apr 1987	Chester R.	Kent Co., DE	39°13'–39°15'N 75°41'–75°43'E
Cod Creek	17 Apr 1987	Nanticoke R.	Sussex Co., DE	38°31'–38°33'N 75°40'–75°42'E
Butler Mill Branch	16 Apr 1987	Nanticoke R.	Sussex Co., DE	38°31'N 75°39'–75°40'E
Chapel Branch	16 Apr 1987	Nanticoke R.	Sussex Co., DE	38°38'–38°40'N 75°39'E
Garey Mill Pond Branch	15 Apr 1987 16 Dec 1988 ¹	Choptank R.	Kent Co., DE	38°56'N 75°43'–75°46'E
Little Whippoorwill Cr.	16 Jun 1987 ¹ 27 Apr 1988	Red R.	Logan Co., KY	36°44'–36°46'N 86°50'–86°53'E
Sinking Creek	27 Apr 1988	Red R.	Logan Co., KY	36°42'–36°43'N 86°47'–86°51'E
Dry Fork Creek	27 Apr 1988	Red R.	Logan Co., KY	36°46'–36°43'N 87°59'E
Cane Creek	19 Jun 1987	Cumberland R.	Putnam Co., TN	36°03'–36°10'N 85°31'–85°37'E
Harid Creek	1 May 1987 2 Feb 1988 ¹	Black Warrior R.	Tuscaloosa Co., AL	33°11'N 87°39'–87°41'E
Jay Creek	30 Apr 1987 2 Feb 1988 ¹	Black Warrior R.	Tuscaloosa Co., AL	33°14'N 87°26'E

¹ Samples examined for sex ratio only; others for sex, length, and age.

streams throughout the Atlantic slopes of the U.S.A. and as far west as Missouri and Arkansas (Rohde & Jenkins 1980), has not been subjected to TFM treatment. Furthermore, since the physical and chemical properties of streams inhabited by lampreys can be expected to vary greatly, this study will also investigate the influence of several other environmental factors on the sex ratio of least brook lamprey larvae.

Although a relationship between density and sex ratio may suggest ESD in lampreys, it does not preclude density-dependent differences between the sexes with respect to mortality or recruitment to the adult population (Hardisty 1961a). The sex ratio of each age class will therefore be determined: if there are sex-specific differences in mortality, for example, a progressive decrease in the proportion of one sex with age is expected. Age in lampreys has traditionally been estimated from length-frequency distributions based on a large number of animals. Although this technique is satisfactory for younger larvae, the cumulative effect of individual variations in growth results in an overlapping of the length distributions of the older age classes (Potter 1980). In the present study, length-frequency age classes are consequently compared to ages determined from statoliths, structures analogous to the

teleost otolith (Medland & Beamish 1987, Beamish & Medland 1988).

If ESD in least brook lampreys is indicated, its possible adaptive significance will be investigated. Environmental sex determination has been proposed, for example, as a tactic for ensuring that an individual of a relatively large size will become the sex in which the rewards for being large are greatest (Conover 1984). If such is the case in least brook lampreys, the following is expected: sexual dimorphism with respect to size; a relationship between growth and the environmental variable to which sex determination is sensitive; and consequently, a relationship between sex ratio and growth.

Methods

Least brook lamprey larvae were collected using electrical fishing gear from the substrate of 12 streams in Maryland, Delaware, Kentucky, Tennessee, and Alabama between April 1987 and December 1988 (Table 1). Identification to species (Vladykov & Kott 1980) was confirmed with adults collected from 8 of the streams. Chemical and physical characteristics of the streams were measured (Table 2), and annual thermal units, expressed as °C days, were estimated for these or nearby streams (US De-

Table 2. Density estimates and physicochemical data for streams from which least brook lampreys were collected. The area given is the product of the length of the stream surveyed and its average width. Density ratings are: 1 = fewer than 0.01 larvae m⁻²; 2 = 0.01–0.05; 3 = 0.05–0.10; 4 = 0.10–0.30; 5 = 0.30–0.50 larvae m⁻²; an asterisk indicates a qualitative estimate only. Values for pH and total hardness represent 1–3 measurements throughout the year.

Stream	Area (m ²)	Density		Annual thermal units (°C days)	pH	Total hardness (mg l ⁻¹ as CaCO ₃)
		larvae m ⁻²	rating			
Unicorn	3645	0.044	2	6275	7.26	60
Jordan			3*	6275	7.22	66
Cod	225	0.251	4	5480	6.57	39
Butler Mill			4*	5480	6.48	39
Chapel			4*	5480	6.52	46
Garey Mill	2700	0.078	3	5088	6.86	35
L. Whippoorwill	22500	0.003	1	5775	7.71	260
Sinking	6000	0.009	1	5775	7.98	238
Dry Fork	24750	0.003	1	5775	8.17	215
Cane	400	0.462	5	5012	7.76	157
Harid	500	0.072	3	5850	7.56	51
Jay	1215	0.148	3	5850	6.92	19

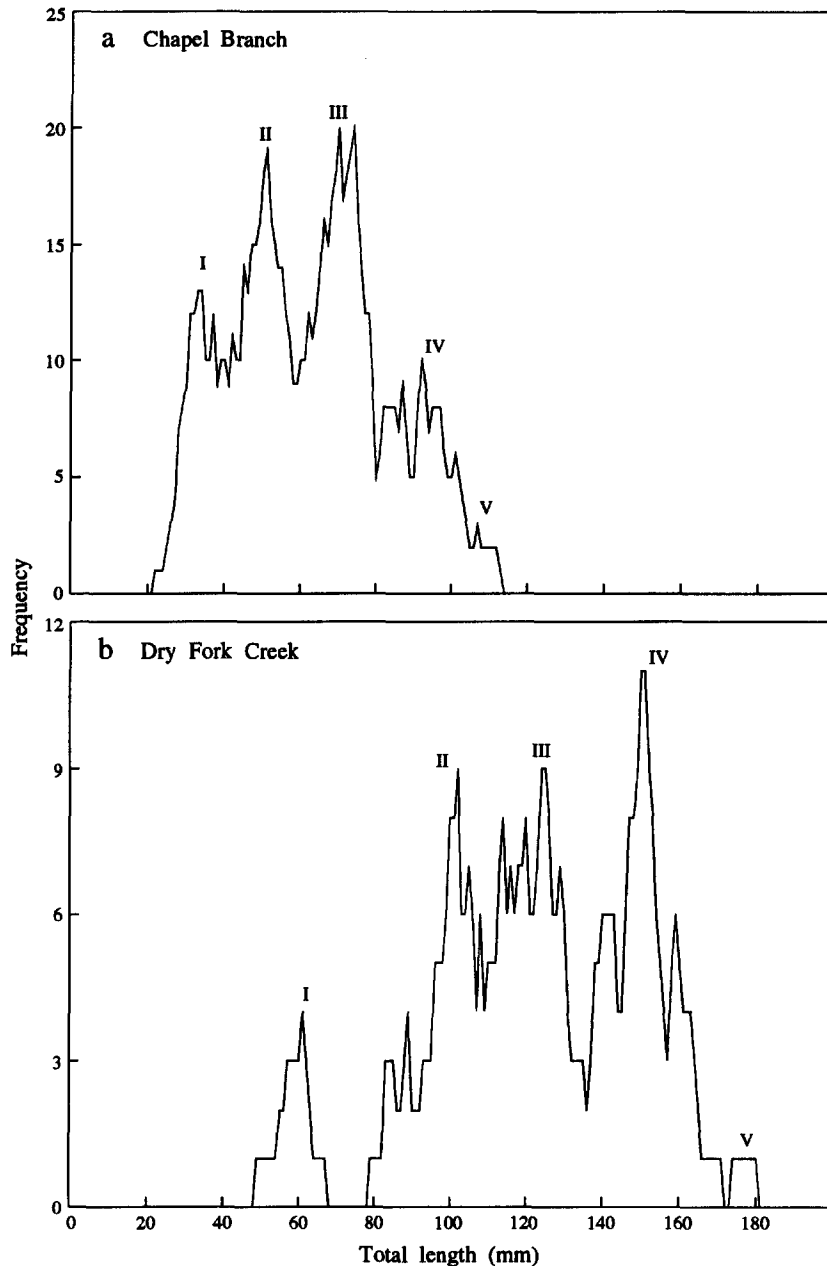


Fig. 1. Age class discrimination from length-frequency distributions for: a – Chapel Branch ($n = 123$); and b – Dry Fork Creek ($n = 71$). Age class frequency peaks, and hence modal lengths, are indicated by the position of the respective Roman numerals. The lowest points between peaks demarcate the age classes; the length range of each age class is presented in Figure 2.

partment of Commerce, National Technical Information Service; Water resources data for MD, DE, KY, TN, and AL water year 1987). The average width of the 12 streams ranged from 2.5 to 10.0 m, and the length of stream surveyed varied depending on the number of larvae collected. In most streams,

distances of 75–675 m yielded large numbers of lamprey larvae; only in Little Whippoorwill and Dry Fork Creeks were distances in excess of 2 km necessary to collect samples of sufficient size. Relative population density was estimated from the number of larvae collected per m^2 of substrate (Ta-

ble 2), and the densities were ranked on a scale of 1 (lowest) to 5 (highest) to allow for the inclusion of 3 streams where sampling area was imprecisely known. The relative density rating (D_r) was directly related to the logarithm of actual density (D_a , larvae m^{-2}) in the streams where the latter was available; the regression equation (\pm SE) is:

$$D_r = 1.7 \pm 0.2 \log D_a + 4.9 \pm 0.3$$

($n = 9$, $p < 0.0001$).

Immediately after capture, all larvae were killed by an overdose of tricaine methanesulfonate (> 100 mg l^{-1}). Length was measured to the nearest 1 mm, and the larvae were frozen for later removal of the statoliths (Beamish & Medland 1988). Larvae were not preserved in formalin because it promotes dissolution of these calcareous structures. After thawing, length and weight of all larvae were recorded to the nearest 1 mm and 1 mg, respectively, and these measurements were used throughout the study unless otherwise indicated. The relationship between fresh length (L_f) and thawed length (L_t), both in mm, was determined for larvae from 6 populations and is described by the regression (\pm SE):

$$L_f = 1.03 \pm 0.003 L_t + 1.13 \pm 0.42$$

($n = 343$, $p < 0.0001$).

Statoliths were removed from the otic capsules, stored in immersion oil for at least 10 days to intensify the banding pattern, and the number of annuli was subsequently counted under a dissecting microscope. Each statolith was aged independently 2–3 times by the same individual, and was numerically coded so that the estimation of age was not biased by the length of the larva or by an age previously assigned. Annuli did not form in larvae from some populations, presumably due to a relatively constant growth rate throughout the year (Beamish & Medland 1988). For age class discrimination in these populations, length-frequency distributions were constructed by taking a sliding average over 7 mm to enhance the frequency peaks. Modal lengths were assigned to each age class and, from the logarithmic relationships between length and weight for each stream, a modal weight was also assigned. To

determine the degree to which length-frequency analysis was reliable, age classes were similarly estimated in populations where statolith annuli were apparent, and the 2 techniques were compared.

The sex of least brook lamprey larvae could be distinguished in all individuals at least 55 mm in length. Two 5-mm long segments were taken from the midregion of each individual, fixed in 5% formalin for a minimum of 48 hours, and embedded in paraffin. The embedded tissues were sectioned to a thickness of 8 μm , stained with Harris' hematoxylin and eosin (Drury & Wallington 1980), and sex was determined following histological examination.

To evaluate postlarval sex ratios and sex-specific differences in body size, metamorphosing and adult least brook lampreys were collected from 8 of the 12 streams, as described previously (Docker & Beamish 1991). Length and weight were measured to the nearest 1 mm and 1 mg, respectively, and sex was determined by visual inspection of the gonad under a dissecting microscope. Only 5 postlarval lampreys were collected from Butler Mill Branch, however, and these were used for identification purposes only.

Data were analyzed using linear regression, analysis of variance (ANOVA), or chi-squared analysis (X^2 , contingency tables) (Statgraphics 1986). Significant deviations in sex ratio from parity were identified from a table of 95% confidence limits for proportions (Beyer 1968). The level of significance used was $p < 0.05$ unless otherwise stated.

Results

Adult least brook lampreys were captured in 7 of the streams between mid-December and late February (Docker & Beamish 1991), and ripe and spent lampreys were found in 2 streams in mid-April. It is thus reasonable to assume that the young-of-the-year larvae emerge between late April and mid-May (Piavis 1971). According to convention (Chilton & Beamish 1982), however, lamprey ages were assigned assuming a January 1 birthdate. That is, the young-of-the-year larvae constitute age class 0 only until December 31.

Statolith annuli were observed in the 7 popula-

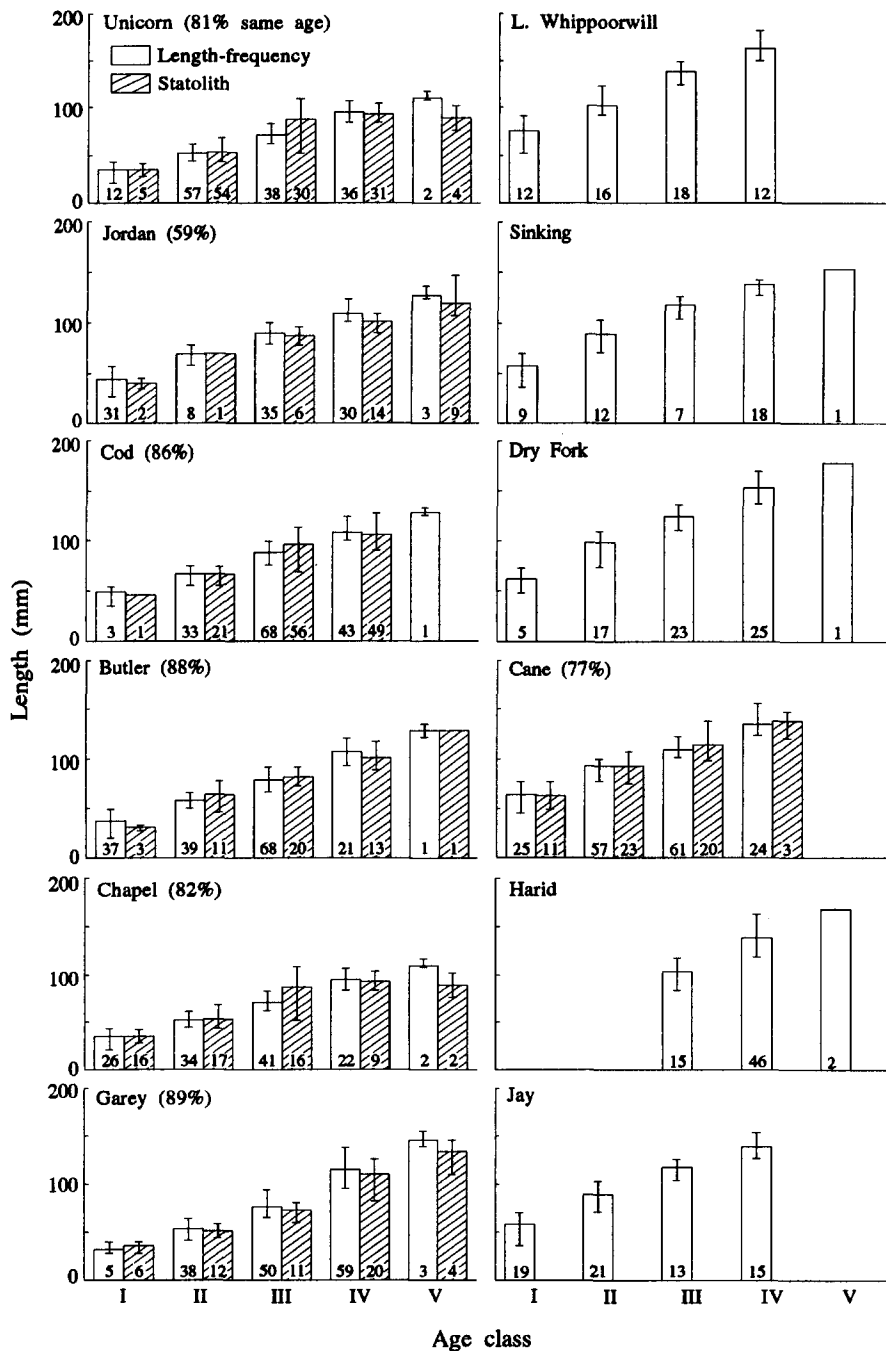


Fig. 2. Modal length of each age class from 12 populations of the least brook lamprey, determined by length-frequency analysis, and compared to mean length of statolith-derived age classes; the percentage of larvae assigned the same age by both techniques is indicated. Sample sizes are given, and vertical bars indicate the length ranges of each age class.

tions from Maryland, Delaware, and Tennessee. Repeated ageing of a given individual resulted in assignment of the same age more than 90% of the time, and the number of annuli increased signifi-

cantly with lamprey total length (linear regression, $p < 0.001$). There were consistently 5 year classes (Fig. 2), although only 4.0% of the 501 larvae that were aged belonged to the fifth year class.

Length-frequency distributions for these populations showed 4 main peaks (e.g., Fig. 1a), which were assumed to be the modal lengths of each of the first 4 age classes; in all but Cane Creek, a minor fifth peak was also evident. Individuals of a given length were assigned an age based on these curves, with the lowest points between the peaks demarcating the age classes. In 82.0% of the larvae aged by both techniques, the length-frequency age corresponded with the statolith annuli number (Fig. 2). Length-frequency curves were fitted to the 5 remaining populations, in which statolith annuli did not form or were indistinct, and these also showed 4 main frequency peaks (e.g., Fig. 1b).

For larvae of age class I, modal length and weight ranged from 32 mm and 80 mg in Garey Mill Pond Branch to 75 mm and 741 mg in Little Whippoorwill Creek (e.g., Fig. 2). Little Whippoorwill Creek was sampled extensively to ensure that a smaller size class had not been overlooked: the smallest larva found was 53 mm and only 5 larvae < 70 mm in length were observed. The modal length and weight of age class IV ranged from 95 mm and 1475 mg in Chapel Branch to 163 mm and 7759 mg in Little Whippoorwill. Modal length and weight corresponded well with mean length and weight of the statolith-derived age classes (Fig. 2).

Size at a given age varied with both population density and the physicochemical characteristics of the stream (linear regression; Table 3). In each age class, length and weight increased significantly with both water hardness and pH (total hardness and pH

were highly correlated; $p = 0.001$), and length decreased significantly with latitude. A decrease in modal length and weight with increasing density was significant in age classes III and IV. Neither length nor weight were significantly related to annual thermal units. Due to their limited numbers, the effect of the environment on the modal length and weight of age class V larvae was not analyzed.

The sex of least brook lampreys could only be identified following a prolonged period of sexual indeterminacy. Prior to sex differentiation, the minute gonad contained only a small number of undifferentiated germ cells (Fig. 3a). Germ cell proliferation, beginning in larvae measuring 35–45 mm in length, subsequently led to the appearance of numerous undifferentiated germ cells and oocytes in both future males and females. In these smaller larvae, identification of the sexes was still ambiguous. By 55 mm total length, however, differentiated females were recognized by the virtual absence of any undifferentiated germ cells and the persistence of a number of nucleated, basophilic oocytes (Fig. 3b). Although differentiation of germ cells into spermatocytes in lampreys does not occur until the end of the larval period (Hardisty 1965a, b), presumptive males were identified by the degeneration of all but a few small oocytes and the persistence of large numbers of undifferentiated germ cells (Fig. 3c).

Although the sex of age class I larvae could often be distinguished in the faster-growing Kentucky populations, sex could not be consistently determined until age class II. Consequently, the sex ratio

Table 3. Levels of significance for regressions between modal length (mm) or weight (mg) at a given age, and stream properties: hardness, pH, latitude, density, and annual thermal units ($^{\circ}$ C days). The sign of each regression coefficient (slope) is given. An asterisk indicates significance at $p < 0.05$.

Factor	Slope	Significance levels (p-values)							
		Age I ¹		Age II ¹		Age III		Age IV	
		mm	mg	mm	mg	mm	mg	mm	mg
Hardness	+	0.002*	< 0.001*	0.002*	< 0.001*	0.001*	< 0.001*	0.002*	< 0.001*
pH	+	0.011*	0.005*	0.002*	< 0.001*	0.003*	0.002*	0.001*	< 0.001*
Latitude	–	0.017*	0.097	0.012*	0.055	0.035*	0.141	0.026*	0.082
Density	–	0.202	0.199	0.109	0.112	0.030*	0.022*	0.022*	0.017*
$^{\circ}$ C days	\pm	0.997	0.797	0.808	0.963	0.716	0.857	0.844	0.885

¹ n = 11, modal length and weight for age classes I and II not available for Harid Creek.

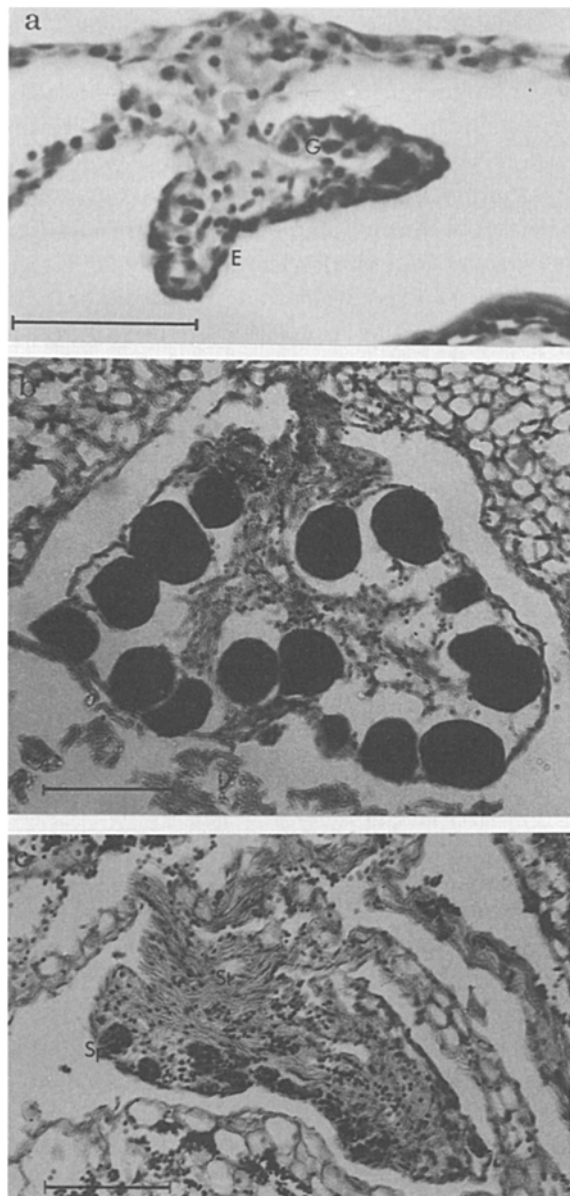


Fig. 3. a – Undifferentiated gonad of a 51 mm least brook lamprey larva, containing germ cells (G) enclosed by a peritoneal epithelium (E). Scale bar = 50 μ m. b – Ovary from a 118 mm larva. Scale bar = 200 μ m. c – Testis from a 104 mm larva, illustrating cysts of presumptive spermatogonia (Sp) and extensive stromal tissue (St). Scale bar = 100 μ m.

of age class I larvae could not be routinely calculated. Given the small sample size of age class V, these larvae were also omitted from overall sex ratio calculations. The sex ratios of age class II to IV larvae varied widely among the 12 streams, from 28.7%

male in Unicorn Branch to 70.9% male in Cod Creek (Table 4). Cod Creek showed a significant preponderance of males (95% CL for proportions), and a significant excess of females was noted in Unicorn, Garey Mill, and Little Whippoorwill Creeks. In each of the 5 streams from which a second collection of larvae was made (Table 1), sex ratios did not differ significantly between collections (X^2 test).

In only Cod Creek and Butler Mill Branch did sex ratios vary significantly among length-frequency age classes (X^2 test): the proportion of males decreased with age in Cod Creek and increased with age in Butler Mill Branch (Table 4). Sex ratios in statolith-derived age classes were similar, but differences were no longer significant (e.g., 86, 65, and 65% male, respectively, in ages II, III, and IV in Cod Creek; 60, 80, and 64% male in ages II–IV in Butler Mill). It is interesting to note that the sex ratio of age class V, although based on a small number of individuals, was overall female-biased. Of a total of 36 age class V larvae, 34.6% were male. In contrast, age classes II to IV averaged 42.3 to 49.3% male.

Sex ratio variations among the populations were related to differences in larval density: the proportion of males increased significantly with relative density (Fig. 4). Sex ratio was not significantly related to water hardness, pH, annual thermal units, or latitude. Sex ratio likewise was not correlated with larval size at a given age: neither overall sex ratio nor the sex ratio of the corresponding age class was related to the modal length or weight of each age class. Sex-specific differences in length and weight were inconsistent, varying among streams and age classes (Table 5). Overall, male and female size differed significantly only among age class V larvae, with females being longer than males (ANOVA).

The sex ratio of postlarval least brook lampreys (Table 6) also varied significantly among streams (X^2 test). Chapel and Unicorn Branches, however, at 79.2 and 75.0% male, respectively, were significantly male-biased (95% CL for proportions). No streams showed a significant excess of females; the smallest proportion of males, 46.2%, was found in Little Whippoorwill Creek. The postlarval sex ratio of each stream was unrelated to the sex ratio of the larval population (linear regression).

The size of the postlarval lampreys likewise var-

ied significantly among streams (ANOVA): the mean length and weight for each stream ranged from 93 mm and 1.5 g in Chapel Branch to 147 mm and 5.9 g in Little Whippoorwill Creek. The smallest and largest postlarval lampreys were 82 and 165 mm, from Chapel and Little Whippoorwill Creeks, respectively. Unlike sex ratio, the length of the postlarval lampreys in a stream was directly related to the modal length of each larval age class (linear regression). In all but Cane Creek, female postlarval lampreys were larger than their male counterparts (Table 6). Accounting for the size variation among streams, the overall sex-specific differences in both length and weight were significant (2-factor ANOVA).

Discussion

Sex ratios of larval least brook lampreys varied widely among the 12 populations examined in this study. The extremes were 29 and 71% male, and the proportion of males increased significantly with population density. A similar relationship between larval sex ratio and adult abundance has been observed in the landlocked sea lamprey (Smith 1971, Purvis 1979). Density-dependent sex determination in lamprey larvae, acting during a prolonged period of sexual indeterminacy, is consequently proposed.

The relationship between sex ratio and abundance has long been observed among spawning adult lampreys, in both the landlocked sea lamprey (Wigley 1959, Hardisty 1961a) and the nonparasitic European brook lamprey, *Lampetra planeri* (Hardisty 1954, 1961a). Hardisty (1961a), however, proposed that there were density-dependent differences

Table 4. Overall sex ratio (percent males) of least brook lamprey larvae in 12 streams, and the sex ratio in length-frequency derived age classes II–IV. Age classes I and V have been excluded from overall sex ratio calculations due to their small sample sizes. Significantly male- or female-biased sex ratios are identified by a superscript ‘m’ or ‘f’ (95% CL for proportions); an asterisk indicates significant variation in sex ratio among age classes (X^2 test). The number of sexed individuals represents only those in which sex could be unequivocally determined.

Stream		Overall		Age II		Age III		Age IV	
		n Sexed	%M	n	%M	n	%M	n	%M
Unicorn	Apr 1987	128	25.8 ^f	54	25.9 ^f	38	23.7 ^f	36	27.8 ^f
	Dec 1988	81	33.3 ^f	13	7.7 ^f	60	38.3 ^f	8	37.5 ^f
	Total	209	28.7 ^f	67	22.4 ^f	98	32.7 ^f	44	29.5 ^f
Jordan		94	48.9	8	50.0	37	45.9	49	51.0
Cod		134	70.9 ^{m*}	32	90.6 ^m	61	65.6 ^m	41	63.4
Butler Mill		102	51.9 [*]	28	32.1	56	57.1	18	66.7
Chapel		96	57.3	34	50.0	40	70.0 ^m	22	45.5
Garey Mill	Apr 1987	123	35.8 ^f	24	37.5	42	35.7	57	35.1 ^f
	Dec 1988	174	43.1			124	45.2	50	38.0
	Total	297	40.1 ^f	24	37.5	166	42.8	107	36.4 ^f
L. Whippoorwill	Jun 1987	16	12.5 ^f	9	11.1 ^f	3	33.3	4	0.0
	Apr 1988	44	36.4	16	31.3	17	41.2	11	36.4
	Total	60	30.0 ^f	25	24.0 ^f	20	40.0	15	26.7
Sinking		47	36.2	12	41.7	17	47.1	18	22.2 ^f
Dry Fork		63	50.8	17	41.2	23	52.2	23	56.5
Cane		142	50.8	56	44.6	60	51.7	26	57.7
Harid	May 1987	61	60.3			15	80.0 ^m	46	54.4
	Feb 1988	47	53.2	3	66.7			44	52.3
	Total	108	57.4	3	66.7	15	80.0 ^m	90	53.3
Jay	Apr 1987	43	37.2	17	47.1	11	45.5	15	20.0 ^f
	Feb 1988	23	65.2	3	66.7	13	61.5	7	71.4
	Total	66	47.0	20	50.0	24	54.2	22	36.4

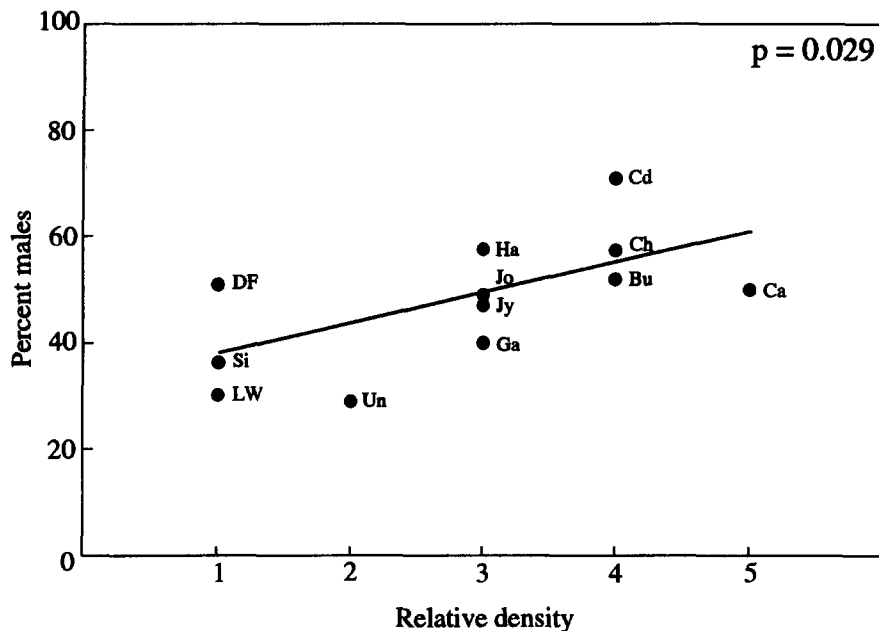


Fig. 4. Relationships between sex ratio (percent males) and relative density of larvae, ranked on an ascending scale of 1 to 5. Bu = Butler, Ca = Cane, Cd = Cod, Ch = Chapel, DF = Dry Fork, Ga = Garey, Ha = Harid, Jo = Jordan, Jy = Jay, LW = Little Whippoorwill, Si = Sinking Creek, Un = Unicorn.

es between the sexes with respect to mortality or recruitment to the adult population. An environmental effect on sex differentiation was dismissed on the finding of approximately equal proportions of male and female larvae in landlocked sea lampreys, European brook lampreys, and the European river lamprey, *Lampetra fluviatilis* (Hardisty 1960). That Hardisty (1960) did not observe skewed sex ratios in larval lampreys may have been due to his examination of only 1 or 2 populations for each species. In the present study, the sex ratios of 8 of the 12 sites examined did not differ significantly from parity, and the sampling of 1 or 2 random sites

may similarly have found equal proportions of male and female least brook lampreys. Furthermore, small changes in density may not produce appreciable differences in sex ratio. For example, the relative density of Cod Creek, in which the bias towards males was greatest, was more than 80 times higher than that of Little Whippoorwill Creek. In the landlocked sea lamprey of the upper Great Lakes, the fluctuations in lamprey abundance have also been of great magnitude (Smith 1971). More extensive surveys of the European brook and river lampreys may yet show support for density-dependent sex determination.

Table 5. Significant sex-specific differences in length and weight of least brook lampreys within each stream and age class; LF and St refer to length-frequency and statolith-derived age classes, respectively.

Stream	Males larger		Stream	Females larger	
	Age class	Variable		Age class	Variable
Chapel	II (LF)	weight	Jordan	V (LF)	length, weight
			Garey	II (LF, St)	length
			Garey	IV (LF)	length, weight
			L. Whippoor.	IV (LF)	length, weight
			Dry Fork	IV (LF)	weight

Furthermore, the present study addressed the issue of sex-specific differences in mortality and recruitment. With regards to differential recruitment, it is both possible and likely that female least brook lampreys metamorphosed at a greater age than males: the oldest larval age class appeared to be female-biased, and females were larger than males among the oldest larvae and the metamorphosing and adult lampreys. That females metamorphose at an older age than males has also been indicated in a number of other lamprey species (e.g., Hardisty 1965b, Purvis 1970, Beamish & Austin 1985, Murdoch et al. 1992). Differential recruitment to the adult stock, however, was not responsible for the differences in larval sex ratios. Similarly, the sex ratio variations were not likely the result of differential mortality between the sexes: sex ratios varied among streams from the time of gonadal differentiation, and remained relatively constant thereafter. Sex ratios differed significantly, but inconsistently, among age classes in only 2 streams. Furthermore, among sea lamprey larvae maintained at various densities for over 3 years, there was no evidence of sex-specific mortality (Docker 1992).

The systematic position of lampreys makes their mode of sex differentiation of interest to comparative biologists, since environmental sex determination has already been demonstrated in some invertebrates and other so-called lower vertebrates. For example, although there have only been a few reports of an effect of population density on sex ratio (Christie 1929, Ellenby 1954, D'Ancona 1950, Lind-

sey 1962), temperature-dependent sex determination is common. Incubation temperature has been found to alter sex ratio in at least 3 teleosts (D'Ancona 1959, Conover & Kynard 1981, Sullivan & Schultz 1986), and is widely known to influence sex differentiation in many reptiles. For example, low incubation temperatures in some turtle species produce males and higher temperatures yield females (e.g., Bull & Vogt 1979, Morreale et al. 1982), whereas in lizards (e.g. Bull 1980) and an alligator (Ferguson & Joanen 1982), the pattern is reversed. In addition, low environmental pH resulted in a predominance of male progeny in 6 species of livebearing teleosts (Rubin 1985). In the present study, lamprey sex ratio was not significantly related to annual thermal units, latitude, or pH.

The adaptive significance of density-dependent sex determination was investigated in the least brook lamprey. It has been suggested that environmental sex determination should be favoured when an environmental factor is more advantageous to one sex or the other (Charnov & Bull 1977). Such control of sex differentiation has been proposed, for example, as a tactic for ensuring that an individual of a relatively large size will become the sex in which the rewards for being large are greatest (Charnov & Bull 1977, Conover 1984). The environmental variables to which sex determination is sensitive may thus act as cues to indicate conditions of favourable growth. For example, an inverse relationship between lamprey density and larval size was significant in the 2 older age classes. Such a decrease in growth at high densities is well-known in

Table 6. Sex ratio of postlarval least brook lampreys, and mean (\pm SE) length and weight of each sex. Lampreys were collected between October and February, and weights were adjusted to correspond to those captured in February (Docker & Beamish 1991). Length and weight after thawing are given for consistency with larval data.

Stream	n	% males	Males		Females	
			Length (mm)	Weight (g)	Length (mm)	Weight (g)
Unicorn Br.	20	75.0	104 \pm 3	2.1 \pm 0.2	106 \pm 4	2.2 \pm 0.2
Chapel Br.	24	79.2	92 \pm 1	1.4 \pm 0.1	98 \pm 3	1.8 \pm 0.2
Garey Mill	25	60.0	103 \pm 2	2.4 \pm 0.1	108 \pm 2	3.1 \pm 0.2
L. Whippoorwill	26	46.2	144 \pm 3	5.7 \pm 0.2	149 \pm 2	6.1 \pm 0.3
Cane Cr.	23	52.2	136 \pm 2	5.6 \pm 0.3	133 \pm 3	5.1 \pm 0.3
Harid Cr.	38	63.2	130 \pm 2	3.7 \pm 0.2	138 \pm 2	4.4 \pm 0.2
Jay Cr.	30	56.7	129 \pm 2	3.6 \pm 0.2	131 \pm 3	3.9 \pm 0.2

teleosts (Allen 1974, Trzebiatowski et al. 1981), and has been observed in other lamprey species (Mallatt 1983, Morman 1987, Murdoch et al. 1992). That the relationship was not consistently significant among the 12 streams examined is likely due to the modifying effects of other environmental factors. Modal length and weight increased with water hardness and pH, and length varied inversely with latitude.

If growth-promoting environmental factors favour differentiation into the sex benefitting most from large size, however, a sexual dimorphism with respect to size would be expected. For example, in parasitic nematodes, where females are universally the larger sex, crowding inhibits growth and results in differentiation of males (Christie 1929, Ellenby 1954). In the Atlantic silverside, *Menidia menidia*, low fluctuating temperatures characteristic of the early breeding season produce a high proportion of females and the higher temperatures representative of late in the season yield an excess of males (Conover & Kynard 1981). Female Atlantic silversides, having a longer growing season, are consequently the larger sex. In nematodes and teleosts, males therefore appear to be penalized less by small size (Charnov & Bull 1977, Conover 1984). For example, whereas gamete production in teleosts may be relatively unconstrained by male body size (Kazamov 1981), fecundity is known to increase with maternal size (e.g., Bagenal 1966, Docker et al. 1986, Hay & Brett 1988). Egg number and female length are likewise correlated in lampreys (Hardisty 1964, Kott 1971, Beamish & Thomas 1983, Docker & Beamish 1991). In the least brook lamprey of the present study, however, female larvae were not consistently larger than the males. In particular, there were no consistent sex-specific differences in size at the approximate time of gonadal differentiation. Females were regularly the larger sex only in the oldest larval age class and in the adult population, presumably due to later metamorphosis as was discussed above.

Furthermore, there was no relationship between larval size at a given age and the sex ratio of the population: streams in which sexual differentiation occurred at relatively large sizes were not consistently female-biased. Although a high proportion of fe-

males was indeed found in Little Whippoorwill Creek where larval size at a given age was greatest, both Unicorn and Garey Mill Pond Branches showed a significant preponderance of females despite the small size of the individuals in the population.

A plausible explanation for the evolution of environmental sex determination in lampreys is consequently lacking. In many reptiles, where temperature-dependent sex determination is well-documented (e.g., Bull & Vogt 1979, Bull 1980, Ferguson & Joanen 1982), its adaptive significance likewise remains elusive (Conover & Heins 1987). It may be that, although fertility is known to increase with body size in females, large body size may also be important to male fitness. Size-specific mortality, for example, may favour large size regardless of sex. Among sea lamprey larvae maintained under controlled conditions for over 3 years, mortality was highest among the smallest of 3 size classes (Docker 1992), and Potter (1980) suggested that a similar pattern exists in nature. Furthermore, the right-skewed length-frequency curves constructed by Manion & Smith (1978) indicate that, even within a single age class, smaller larvae suffer greater mortality.

Further speculation on the possible adaptive significance of environmental sex determination in lampreys clearly requires a better understanding of their ecology. In addition to the above considerations of fertility and survival, for example, future studies should examine the demographics of the spawning stock. For example, highly-skewed sex ratios could lead to frequency-dependent selection for the rarer sex (Charnov & Bull 1977, Conover & Heins 1987). Consequently, the environmental cue to which sex differentiation is sensitive would be expected to be spatially or temporally variable to ensure adequate production of both sexes (Bull 1980, Conover 1984). Mating between individuals from different densities would then result in spawning sex ratios closer to parity.

The extent to which least brook lamprey density is spatially variable within its range of dispersal, however, appears limited. For example, although there is evidence that some sea lampreys do not home to their natal streams (Potter et al. 1974), mat-

ing between nonmigratory least brook lampreys from different streams is unlikely. Furthermore, an unpublished study by F.W.H. Beamish suggests that density and sex ratio may be relatively constant within a given stream. Alternatively, there may be annual differences in population density and sex ratio, and individual or sex-specific differences in age at metamorphosis (Manion & Smith 1978) would result in spawning among lampreys from different age classes. The populations in the present study, however, appeared to show relatively minor annual variations in sex ratio. The 5 populations sampled up to 20 months apart showed little change in sex ratio between collections, and there was little variation among year classes.

Larval sex ratios, however, are not necessarily representative of sex ratios at spawning. In the present study, there was a clear bias towards males among spawning least brook lampreys and, relative to the larval sex ratios, this excess of males was not consistent. Hardisty (1960) found a similar disparity between larval and adult sex ratios, which he has attributed to higher female mortality at some time between metamorphosis and spawning (Hardisty 1961b). Alternatively, it could be that the disproportionate percentage of males at spawning was artificial, due either to small sample sizes or to the time or location of collection. Lamprey sex ratios, for example, have been shown to fluctuate during the spawning season (Zanandrea 1951, Hardisty 1961b, Stier & Kynard 1986), and may be attributed either to differential migration rates between the sexes (Applegate & Thomas 1965) or shorter female residence time on the spawning grounds (Farlinger & Beamish 1984). A better understanding of the relationship between larva and adult sex ratios is crucial to further hypotheses regarding the evolutionary significance of environmental sex determination in lampreys.

In summary, all 3 female-biased populations of the least brook lamprey were characterized by low larval density, and among the 12 geographically-diverse streams, the proportion of males increased significantly with density. Although females appear to metamorphose later than males, the number of age class V larvae was minimal and the observed differences in sex ratio were not the result of differ-

ential recruitment. Likewise, differential mortality between the sexes was unlikely. Rather, the variable sex ratios were established at the time of gonadal differentiation and remained relatively constant over the subsequent 2–3 years of larval life. Although only experimental studies can verify a causal relationship between density and sex ratio, density-dependent sex determination is indicated in the least brook lamprey. Similarly, although the possible adaptive significance of ESD in lampreys remains elusive, further experimental and field studies may shed some light on density-related differences between the sexes.

Acknowledgements

Financial assistance was provided through an operating grant (F.W.H.B.) and postgraduate scholarship (M.F.D.) from the Natural Sciences and Engineering Research Council of Canada. Scientific collecting permits were kindly supplied by Alabama Department of Conservation, Delaware Department of Natural Resources and Environmental Control, Kentucky Department of Fish and Wildlife Resources, Maryland Department of Natural Resources, and Tennessee Wildlife Resources Agency. T.E. Medland performed the statolith ageing, for which we are especially grateful.

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