Environmental Sex Determination in Southern Brook Lamprey, *Ichthyomyzon gagei*

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Sex ratios were determined for 20 populations of southern brook lamprey larvae, *Ichthyomyzon gagei*, collected from throughout much of their range in the southeastern United States. Ratios varied between 9 and 49% males. Differential mortality was an unlikely factor, as sex ratios were similar among age groups within each population. Environmental sex determination is suggested. Sex ratio varied with growth (expressed as length of larvae at specific ages), larval density, pH, and annual mean temperature of the natal stream. Generally, under conditions promoting rapid growth, the percentage of males varied directly with density and inversely with temperature. Where growth was slow, the percentage of males declined as larval density increased, the response being less at low than at high pH. Temperature had little effect when larval growth was slow. The percentage of males declined when growth was rapid under otherwise similar environmental conditions.

Nous avons calculé le rapport des sexes dans 20 populations de lamproie, *Ichthyomyzon gagei*, à l'état larvaire, recueillies dans une bonne partie de leur aire de répartition du sud des États-Unis. Les rapports variaient de 9 à 49 % de mâles. La différence dans le taux de mortalité semble un facteur peu probable, car les rapports des sexes étaient similaires entre les groupes d'âge dans chaque population. On peut proposer que le sexe est déterminé par l'environnement. Le rapport des sexes variait en fonction de la croissance (exprimée par la longueur des larves à des âges spécifiques) de la densité des larves, du pH et de la température annuelle moyenne du cours d'eau natal. En général, dans des conditions favorisant une croissance rapide, le pourcentage de mâles variait en fonction directe de la densité et en fonction inverse de la température. Quand la croissance était lente, le pourcentage de mâles baissait à mesure que la densité de larves augmentait, la réaction étant moins forte à un pH bas qu'à un pH haut. La température avait peu d'effet quand la croissance des larves était lente. Le pourcentage de mâles baissait quand la croissance était rapide dans des conditions environnementales par ailleurs similaires.

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The phylogenetically primitive lamprey (Bardack and Zangerl 1971; Hardisty 1979) are characterized by a long larval period which is spent within the soft substrate of cool-water streams. For much of the larval period the gonads remain undifferentiated (Okkelberg 1921; Hardisty 1965a, 1965b). Parasitic lamprey, those species that have retained the juvenile period during which they feed on the body fluids and tissues of fishes, mature a large number of oocytes (Vladykov 1951; Beamish and Potter 1975). Nonparasitic or brook lamprey have not retained the juvenile period and are less fecund (Vladykov 1951; Abakumov 1966; Beamish and Thomas 1983) than their presumed parasitic ancestors (Hubbs 1925; Hubbs and Trautman 1937; Zanandrea 1959; Potter 1980).

The potential for exponential increase in abundance of the parasitic sea lamprey, *Petromyzon marinus*, was realized soon after their invasion of the upper Great Lakes in the late 1930s (Applegate 1950; Smith and Tibbles 1980). A characteristic of this period of increasing abundance was a preponderance of males in adult populations (Smith 1971). Sea lamprey abundance was reduced precipitously following treatment with the lampricide 3-trifluoromethyl-4-nitrophenol (TFM), and the proportion of male larvae and adults correspondingly declined (Smith 1971; Purvis 1979). Since there is no evidence to suggest that TFM is differentially toxic to male and female lamprey (Manion and Smith 1978), it appears that sex differentiation in sea lamprey may be influenced by population size.

There is little doubt that sex has a genetic basis among the lower vertebrates despite the frequent absence of recognizable sex chromosomes (Yamamoto 1953; Richards and Nace 1978; Hunter et al. 1983; Yamazaki 1983). In at least some taxa among the lower vertebrates, the expression of sex is both variable and labile (Atz 1964; Bull 1980; Yamazaki 1983). Environmental conditions are known to irreversibly alter primary sex differentiation in some gonochoristic species. Egg incubation temperature (Bull and Vogt 1979; Conover and Kynard 1981; Ferguson and Joanen 1982; Mohanty-Hejmadi and Dimond 1986), pH (Rubin 1985), and population density (D'Ancona 1950; Lindsey 1962) have been identified as factors causing environmental sex determination in some taxa of lower vertebrates. Kirpichnikov (1981) suggested that male and female genes in fish are located in many chromosomes and the determination of sex depends on the balance of these genes. Each gene affecting gonad development is thought to be relatively weak in action, and therefore, a change in environmental conditions and the resulting variation in genotype may easily be accompanied by changes in population sex ratio.

Environmental sex determination has been related to population density in least brook lamprey, *Lampetra aepyptera*, with

the proportion of males increasing directly with the species' abundance (M.F. Docker and F.W.H. Beamish, Department of Zoology, University of Guelph, Guelph, Ont., personal observation). The present study examined 20 populations of southern brook lamprey, *Ichthyomyzon gagei*, from much of their geographical distribution (Lee et al. 1980) for evidence of environmental sex determination.

Materials and Methods

Southern brook lamprey were electrofished from streams in which it was the only species of lamprey. Identification was confirmed from diagnostic taxonomic characteristics displayed by adults collected from each stream (Hubbs and Trautman 1937). Some streams were sampled for lamprey only once, and others several times over a period of years (Table 1). Generally, lamprey were collected from more than one location in a stream or, when access was extremely difficult, from a length of stream in excess of 1 km. The area of substrate electrofished was measured to allow calculation of larval density.

Several broad-spectrum indicators of water quality were usually measured each time a stream was sampled. Total hardness (EDTA) and alkalinity (pH 4.5) were measured directly in the field (APHA 1989). Conductivity and pH were measured, also in the field, with probes that were regularly calibrated. Annual mean temperatures were estimated from temperature records for these or nearby streams (U.S. Department of Commerce, National Technical Information Service, Water Resources Data for Alabama, Arkansas, Louisiana, Mississippi, and Texas, water year 1987).

Immediately after capture, all animals were either anaesthetized in tricaine methanesulfonate (1% MS222 by weight) or killed by an overdose of the anaesthetic and total length measured to the nearest millimetre. This information was used to construct length–frequency distributions as one method of estimating population age structure. Larvae that were killed were frozen for subsequent determination of sex and removal of statoliths, structures analogous to teleost otoliths and similarly used for age determination (Beamish and Medland 1988).

In the laboratory, frozen lamprey were thawed, again measured for total length, statoliths removed, and the gonads examined. Lengths of thawed larvae were adjusted to correspond to live length on the basis of the following regressions:
For larvae 25–75 mm in thawed length:

$$L_l = 1.04L_t - 0.95 \qquad (n = 150)$$

For larvae 76–200 mm in thawed length:

$$L_l = 1.10L_t - 3.50 \qquad (n = 150)$$

where L_l is total length of live larvae and L_t is total length of thawed larvae. All lengths in this study are reported as live total length.

Statoliths were removed from the otic capsules of larvae and stored in immersion oil for 10–30 d to intensify the banding patterns (Medland and Beamish 1987). Each statolith was examined under a dissecting microscope and, when annuli were recognizable, aged independently two or three times. Statoliths were numerically coded so that the estimation of age was not biased by the length of that individual or by an age previously assigned. The same age was almost always assigned on repeated examination of the same statolith. Where it was not possible to age individuals, a length–frequency distribution was constructed for the population by taking a moving average over 7 mm to enhance the frequency peaks (Hardisty 1961) and the age of individuals assigned on the basis of their length.

TABLE 1. Locations of streams from which southern brook lamprey were captured and collection dates. When only a single collection was made from a stream, the month is given.

State	County or parish	Stream	Date
Alabama	Tuscaloosa	Binion Cr.	1987–90
Alabama	Macon	Choclafaula Cr.	1980–91
Alabama	Covington	Eden Cr.	1989–90
Alabama	Covington	Teel Cr.	1989–90
Alabama	Escambia	Hell Hole Cr.	1987–91
Arkansas	Hot Springs	Keisler Cr.	April 1989
Arkansas	Hot Springs	Thomas Cr.	April 1989
Arkansas	Saline	South Fork Saline R.	April 1989
Arkansas	Saline	Ten Mile Cr.	April 1989
Louisiana	Tangipahoa	Beaver Cr.	April 1990
Louisiana	Tangipahoa	Spring Cr.	April 1989
Louisiana	Tangipahoa	Terry's Cr.	April 1989
Louisiana	Grant	Big Cr.	April 1990
Louisiana	Grant	Clear Cr.	April 1989
Louisiana	Grant	Dry Prong Cr.	April 1989
Louisiana	Grant	Dyson Cr.	April 1990
Mississippi	Simpson	Uspoha Cr.	April 1987
Mississippi	Perry	Water Prong Cr.	1987–90
Texas	Nacagdoches	Legg Cr.	April 1989
Texas	Tyler	Little Cypress Cr.	April 1989

Sex of the larger larvae was determined by visual internal inspection of the gonad under a dissection microscope. Histological examinations were conducted on all of the smaller larvae and, periodically, on the larger lamprey to confirm the identification made by visual inspection. Histological examinations were based on two 5-mm-thick cross-sections taken from the midregion of an individual, fixed in 5% formalin for a minimum of 48 h, and embedded in parafin. The subsequent 8-μm-thick sections were stained with hematoxylin and eosin (Willey 1971) and sex was determined under a compound microscope (Docker 1992).

Results

Southern brook lamprey spawn in the spring (Raney 1952; Dendy and Scott 1953; Beamish 1982). Observations on the population in Choclafaula Creek, Alabama, over an 11-yr period indicate that spawning commences early in April and is complete within about 4 wk. Other populations in Alabama have been observed to follow a similar spawning schedule. In Louisiana, spawning activity among lamprey from the seven streams sampled was most intense during mid-April. Spawning appears to reach a peak during early to mid-April in Mississippi and is complete by late April. Southern brook lamprey spawn about a week earlier than this in those streams examined in Arkansas and Texas. In general, spawning activity appears to be most intense during mid-April throughout the geographic region examined in this study. It is probable that eggs hatch within 2–3 wk after spawning (Piavis 1971). For the purpose of this study, May 1 was assigned as the southern brook lamprey's birthdate in all streams.

The assignment of age from annuli on statoliths was possible only for the population of brook lamprey from Binion Creek. In other populations, annuli were not recognizable and age was assigned from length–frequency distributions. For a sample of

TABLE 2. Relationship between total length and age in southern brook lamprey larvae. In the linear growth equation, L is modal total length (millimetres) of larvae and A is age (months). All correlation coefficients (r) were significant at $P < 0.05$ for the sample sizes, (n) used to derive the equations.

Stream	Total no. of larvae	Growth equation $L = a + bA$			
		a	b	n	r
Binion Cr.	211	55.5	2.1	8	0.990
Choclafaula Cr.	4742	22.3	2.7	34	0.998
Eden Cr.	239	40.6	3.1	8	0.983
Teel Cr.	174	23.3	2.3	4	0.991
Hell Hole Cr.	611	49.7	1.9	12	0.999
Keisler Cr.	155	5.6	2.6	4	0.996
Thomas Cr.	153	20.4	2.4	4	0.999
South Fork Saline R.	76	8.8	2.8	4	0.987
Ten Mile Cr.	217	39.5	2.5	4	0.998
Beaver Cr.	73	55.1	2.6	4	0.989
Spring Cr.	91	59.8	2.3	4	0.984
Terry's Cr.	138	39.8	2.7	4	0.985
Big Cr.	152	12.7	3.2	4	0.998
Clear Cr.	189	18.5	2.5	4	0.999
Dry Prong Cr.	83	6.5	3.0	4	0.997
Dyson Cr.	131	31.2	2.3	4	0.997
Uspoha Cr.	269	23.1	2.2	4	0.999
Water Prong Cr.	260	21.0	2.0	4	0.998
Legg Cr.	171	24.4	2.4	4	0.999
Little Cypress Cr.	181	27.4	2.4	4	0.997

larvae collected from Binion Creek in late April 1988, mean total length (±SD) of larvae aged 24, 36, and 48 mo, on the basis of statolith banding patterns, was 79.0 ± 20.2 (n = 7), 110.5 ± 12.6 (n = 26), and 134.7 ± 9.2 mm (n = 22). These were similar to the modal lengths of 74, 101, and 132 mm estimated from the length–frequency distribution for the same collection of larvae from Binion Creek.

A growth equation was derived for larvae from each stream or population based on modal lengths and age. For most populations, it was not possible to compare growth among age classes. However, with the extensive data base for Choclafaula Creek, length throughout larval life was compared among six successive age classes beginning in 1976 (SAS 1989; general linear model). Lengths for each age group (i.e., 12, 24, 36 mo) were not significantly different among the six age classes ($F_{5,21}$ = 1.00, $P > 0.05$). Hence, all modal lengths for larvae of a given age from Choclafaula Creek were pooled and a single growth equation derived:

$$L = 22.3 + 2.7A \qquad (n = 34; r = 0.990)$$

where L is live total length (millimetres) of larvae and A is age (months).

For each of the other populations, one to three collections of lamprey were made. Length–frequency curves indicated a larval life of 4.25 yr (51 mo) for most animals in all streams, with the probability that a small number of animals complete the larval interval 1 yr earlier. Modal lengths from the length–frequency distributions were used to derive growth equations (Table 2) which were used to calculate lengths at 12, 24, 36, and 48 mo.

Sex of southern brook lamprey could be determined for most larvae older than 11 mo. Prior to sex differentiation, gonads contained only small numbers of undifferentiated germ cells. The ovary of the differentiated female was characterized by the appearance of a number of nucleated basophilic oocytes (Docker 1992). Males could be recognized only by the absence of oocytes, since differentiation of germ cells into spermatocytes does not occur in larvae.

Sex ratio varied among populations from 9 to 49% males (Table 3). Sex ratio was not significantly different from parity ($P > 0.05$) in five of the populations. In the remaining populations, females were significantly the more abundant sex. Generally, the overall bias in sex ratio for a population was maintained across all age groups and was especially evident where numbers were large (Fig. 1). In most populations, mean length of females in the oldest age group was greater than that of males. However, differences were significant only in Teel and Keisler creeks and in one collection from each of Choclafaula and Water Prong creeks (t-statistic; $P < 0.05$). Among the younger age groups, there was neither statistical support nor a pattern of difference in length between the sexes.

The percentage of males within a population was not related to larval density ($P > 0.05$) but varied inversely with modal total length at a given age, the latter being a measure of growth. Modal total lengths were calculated from the equations in Table 2. The relationship between percentage of males and length-at-age among populations was linear and significant ($P < 0.05$) and is described by the following equations:

$$S_m = 80.3 - 0.6L_2 \qquad (n = 20; r = 0.663)$$
$$S_m = 88.2 - 0.5L_3 \qquad (n = 20; r = 0.554)$$
$$S_m = 87.6 - 0.4L_4 \qquad (n = 18; r = 0.531)$$

where S_m is the percentage of male larvae within a population and L_2, L_3, and L_4 are the modal total lengths of larvae at 24, 36, and 48 mo, respectively.

The range in concentration of total hardness, alkalinity, and conductivity within and among streams was not large (Table 4), and for each identity, it was not possible to demonstrate a significant relationship with the percentage of males. Similarly, the percentage of males was not significantly correlated with the annual mean temperature estimated for each of the 20

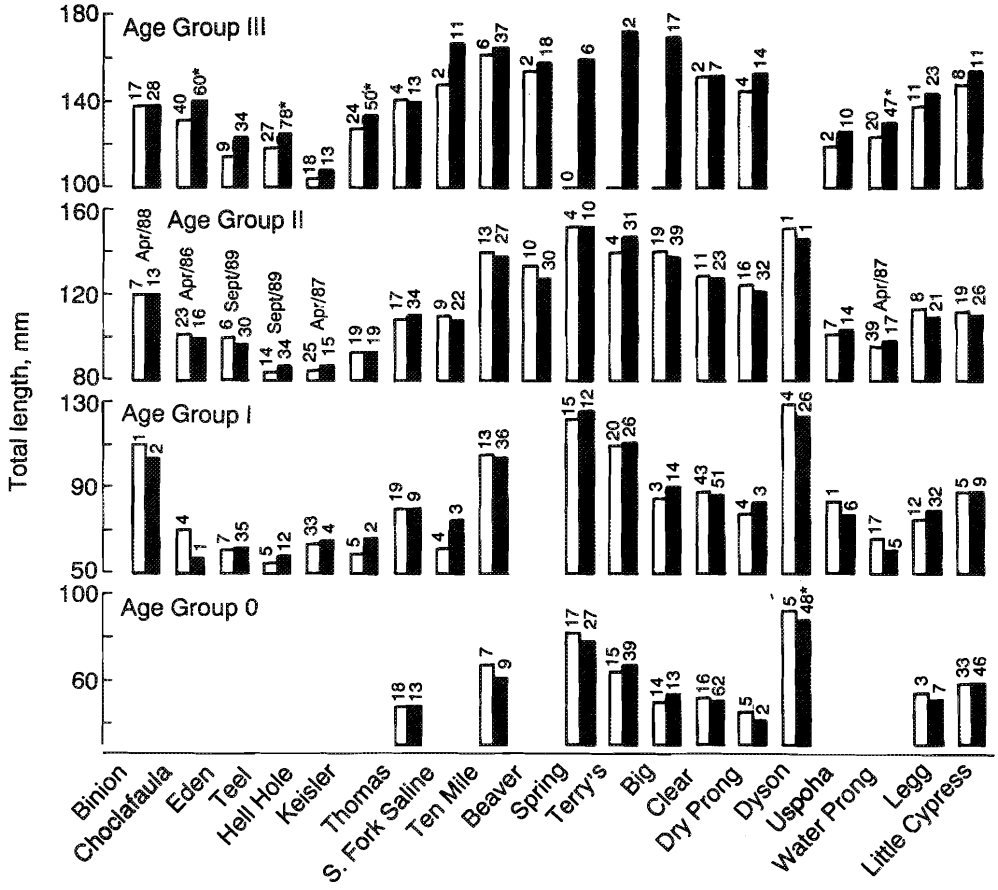

FIG. 1. Mean total length of male (open bars) and female (solid bars) larvae by age group. Sample size is indicated by the number above each bar. Significant differences in length ($P < 0.05$) between sexes within an age group are indicated with an asterisk. Two or more collections of larvae were made from each of the streams in Alabama and from Water Prong Creek in Mississippi (Table 1), but the results of only one representative collection are reported in the figure. Sampling dates for the other streams are as reported in Table 1.

TABLE 3. Sex ratios of southern brook lamprey populations and density of larvae based on surface area of substrate electrofished. Significant deviations in sex ratio from parity, based on 95% confidence limits (CL) for proportions (Beyer 1968), are identified with asterisks.

Stream	No. of larvae sexed	Sex ratio		Larval density (no.·m^{-2})
		% ♂	95% CL	
Binion Cr.	120	37*	28–45	0.17
Choclafaula Cr.	1707	41*	35–47	0.68
Eden Cr.	121	19*	12–27	0.29
Teel Cr.	170	27*	26–35	0.13
Hell Hole Cr.	219	49	44–57	1.75
Keisler Cr.	119	40*	32–48	0.04
Thomas Cr.	127	46	37–55	0.18
South Fork Saline R.	51	29*	17–43	1.90
Ten Mile Cr.	148	26*	19–34	1.55
Beaver Cr.	60	20*	11–32	0.26
Spring Cr.	91	40	30–51	—
Terry's Cr.	137	28*	21–37	—
Big Cr.	119	30*	22–39	1.52
Clear Cr.	185	39*	32–47	1.20
Dry Prong Cr.	80	36*	27–47	0.99
Dyson Cr.	95	9*	4–17	0.50
Uspoha Cr.	40	38	23–54	—
Water Prong Cr.	219	45	40–53	1.13
Legg Cr.	117	29*	21–39	0.24
Little Cypress Cr.	157	41*	33–49	0.23

TABLE 4. Physicochemical characteristics (mean ± SD) of the streams from which southern brook lamprey were captured. n = sample size.

Stream	Annual mean temperature (°C)	n	pH	Conductivity (μS)	Alkalinity ($mg \cdot L^{-1}$)	Total hardness ($mg \cdot L^{-1}$)
Binion Cr.	20.9	5	7.1 ± 0.1	22 ± 5	10 ± 4	12 ± 5
Choclafaula Cr.	14.6	14	7.2 ± 0.2	127 ± 18	115 ± 11	97 ± 9
Eden Cr.	20.9	4	7.0 ± 0.3	68 ± 14	34 ± 4	34 ± 4
Teel Cr.	20.9	4	7.2 ± 0.2	94 ± 22	47 ± 10	50 ± 11
Hell Hole Cr.	19.1	7	6.8 ± 0.2	21 ± 3	10 ± 3	13 ± 4
Keisler Cr.	20.0	3	6.3 ± 0.2	39 ± 10	9 ± 2	12 ± 3
Thomas Cr.	15.5	3	6.4 ± 0.4	37 ± 9	13 ± 2	14 ± 3
South Fork Saline R.	15.5	2	7.1	134	44	51
Ten Mile Cr.	19.1	3	7.2 ± 0.2	86 ± 24	28 ± 5	35 ± 10
Beaver Cr.	17.5	2	6.5	43	15	16
Spring Cr.	17.5	3	6.6 ± 0	47 ± 4	17 ± 9	16 ± 2
Terry's Cr.	20.0	4	6.8 ± 0.1	40 ± 4	16 ± 6	13 ± 3
Big Cr.	17.5	3	7.0 ± 0.2	46 ± 2	14 ± 4	12 ± 1
Clear Cr.	20.0	3	7.1 ± 0.3	47 ± 8	22 ± 5	12 ± 3
Dry Prong Cr.	20.0	1	6.2	32	18	12
Dyson Cr.	20.0	2	6.8	33	15	12
Uspoha Cr.	17.9	4	6.6 ± 0.2	36 ± 8	17 ± 3	13 ± 3
Water Prong Cr.	17.9	3	6.0 ± 0	21 ± 8	9 ± 2	9 ± 2
Legg Cr.	18.4	2	7.1	113	34	35
Little Cypress Cr.	18.1	2	7.0	87	27	37

streams. Percentage of male lamprey decreased as pH increased between 6.0 and 7.2. However, the correlation between percentage of males and pH was not significant ($P > 0.05$). When the sex ratio for larvae from Beaver Creek was excluded, on the assumption a bias may have occurred due to the small sample ($n = 60$), the regression was significant ($P < 0.05$):

$$S_m = 116.3 - 12.1p \qquad (r = 0.443)$$

where p represents pH.

The combined effects of larval growth and density and stream temperature and chemistry on sex ratio were examined by multiple regression analysis (SAS 1989; REG procedure) and expressed by the regression

$$S_m = 175.5 - 2.4L_2 + 0.7L_2 \cdot D + 0.3L_2 \cdot p - 3D \cdot p + 5.2TD - 0.8 \cdot 10^{-1} L_2 \cdot T$$

where L_2 is the total length of larvae at 24 mo, T is the annual mean stream temperatures (degrees Celsius), and D is larval density (number per square metre). Variables were retained in the model if their regression coefficients had t values that were significant at $P < 0.15$:

Variable	Regression coefficient	SE	Student's t
Intercept	175.5	±57.3	3.06[a]
L_2	2.4	±1.4	1.67[b]
$L_2 \cdot D$	0.7	±0.5	1.65[b]
$L_2 \cdot p$	0.3	±0.1	2.68[a]
$D \cdot p$	21.3	±8.2	2.57[a]
$T \cdot D$	5.2	±1.7	3.10[a]
$L_2 \cdot T$	0.8×10^{-1}	±0.2	3.71[a]

[a]Significant at $P < 0.05$.
[b]Significant at $P < 0.15$.

The regression's F value is 3.66 ($P < 0.05$) and the coefficient of multiple determination (R^2) is 0.69. Neither skewness nor kurtosis is significant at $P > 0.05$.

The equation predicts that among populations of southern brook lamprey from streams characterized by an annual mean temperature of 15°C and pH 7.2, the percentage of males can be expected to decline as larval density increases provided individual growth rates are slow (Fig. 2). When growth is rapid, the percentage of males changes very little with larval density. The percentage of males is, at each larval density, less when growth is rapid than when it is slow. At pH 6.0 and 15°C, the equation predicts that among populations in which individuals are slow growing, the percentage of males will decline slightly as larval density increases (Fig. 2). In marked contrast, the percentage of males increases with larval density when individual growth is rapid. At pH 6.0, as is the case at 7.2, the percentage of males, at a given larval density, varies inversely with larval growth. It is noteworthy at pH 6.0 that the equation predicts the absence of males from populations in which growth is very high but larval density is low.

Southern brook lamprey are found typically in streams in which the annual mean temperature ranges from about 15 to 20°C. Over this range the regression predicts relatively little effect of temperature on population sex ratio when growth is slow and pH is low. However, in populations that exhibit rapid growth, the percentage of males varies inversely with temperature. Thus, among populations in which the modal length of larvae at 24 mo is 65 mm from streams of pH 6.5 with annual mean temperatures in the range of 15–20°C, the percentage of males is approximately 53% (Fig. 3). Under conditions that promote more rapid growth, the percentage of males decreases linearly, with the rate of decline being greater at 20 than at 15°C. Thus, in populations in which the modal length of larvae at 24 mo is 100 mm, the percentage of males is reduced to about 20 and 6 at 15 and 20°C, respectively.

Discussion

Variations in sex ratios of adult lamprey have been reported for both parasitic (Wigley 1959; Hardisty 1961; Beamish and Potter 1975; Stier and Kynard 1986) and nonparasitic species (Dean and Sumner 1898; Hardisty 1954, 1961; Purvis 1970). Early in his investigations, Hardisty (1954) suggested that the

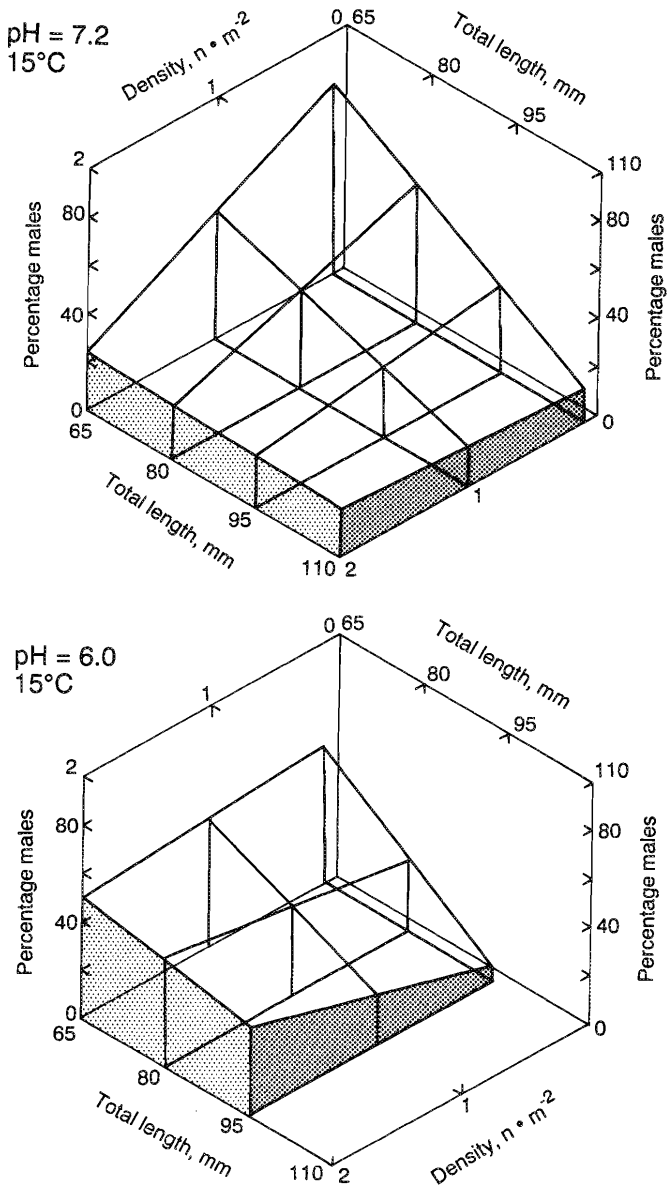

FIG. 2. Predicted population sex ratio, as percentage of males, in relation to larval density and growth, the latter as total length at 24 mo of age.

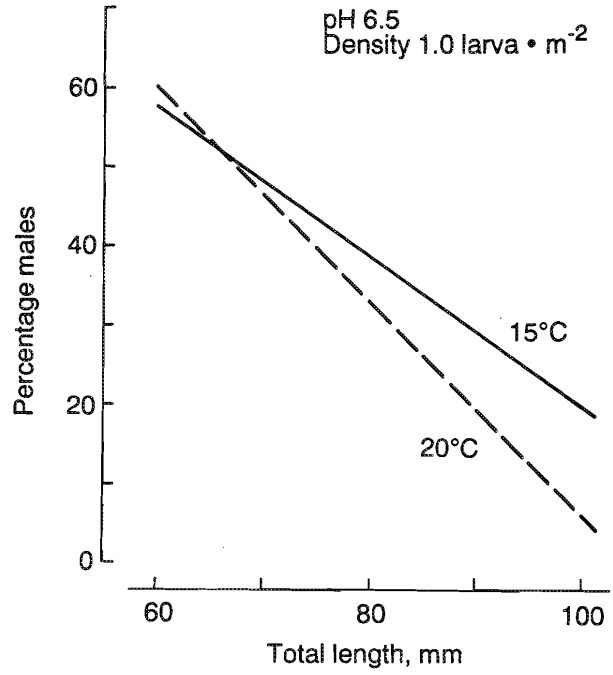

FIG. 3. Influence of annual mean stream temperature and larval growth (length at 24 mo) on population sex ratio when density of larvae is $1 \cdot m^{-2}$ and ambient pH is 6.5.

variation in sex ratio of adult brook lamprey, *Lampetra planeri*, might be due to the effect of the environment on the course of gonadal differentiation. In a subsequent study, Hardisty (1960) examined small collections of *Lampetra fluviatilis* larvae from two streams, a small sample of sea lamprey larvae from one stream, and a large sample of *L. planeri* larvae from a single stream and found sex ratios to vary only between 42 and 49% males. He concluded that these differences from parity were not sufficient to support his earlier view of environmental sex determination. The direct association between percentage of adult male lamprey and their respective population size was attributed to differences in adult recruitment or sex-related mortality. Hardisty's (1960) conclusion was, in part, based on the assumption that most mortality after the prolarva interval occurs during metamorphosis or during the period of sexual maturity. Research currently underway on mortality of southern brook lamprey indicates quite significant rates of instantaneous natural mortality during larval development in some streams.

Long-term studies of the landlocked sea lamprey in the upper Great Lakes have found sex ratios to vary widely with relative abundance. When abundance was high following their invasion of the upper Great Lakes, adult males typically composed 60%, and in some years as much as 70%, of the population (Heinrich et al. 1980). As lamprey numbers were dramatically reduced following the application of TFM, the proportion of adult and larval males correspondingly declined and a predominance of females emerged (Smith 1971; Purvis 1979; Heinrich et al. 1980). No evidence is available to suggest that TFM is differentially toxic to the two sexes (Purvis 1979; NRC 1985). Sea lamprey populations naturally characterized by low densities similarly show an excess of females (Purvis 1979). Recently, M.F. Docker and F.W.H. Beamish (personal observation) found that sex ratios of larval least brook lamprey varied directly with relative abundance from 24 to 71% males. These patterns of increase in the proportion of larval and adult male lamprey with population size are similar to that found for larval southern brook lamprey except among populations where individual growth was slow in which case the pattern was reversed.

Somatic growth of southern brook lamprey larvae is, for most populations, similar throughout the year. Extensive collections of larvae from Choclafaula Creek demonstrated little seasonal variation in growth reflecting the combined effects of only moderate temperature changes and fluctuating stream discharge (Beamish 1982). Accordingly, statolith growth in larvae from Choclafaula Creek was uniform and annuli did not form (Beamish and Medland 1988). Failure to recognize statolith banding patterns in other populations presumably also indicates even growth throughout the year. Only in Binion Creek was seasonal growth sufficiently variable to provide recognizable annuli in statoliths.

Support for environmental sex determination is strengthened by observations in this study and in that by Docker and Beamish of similar sex ratios among age groups within streams. This

observation refutes earlier suggestions of differential mortality between the sexes accounting for skewed population sex ratios. A more appealing mechanism is suggested by the observations made by Epple et al. (1982). They found that circulating steroids in larval sea lamprey responded to crowding. Of particular interest to the argument of environmental sex determination was the direct association between larval density and testosterone concentration. It is worthy of note that Docker (1992) was unable to direct the course of gonadal differentiation by exposing lamprey to any of several gonadal steroids including testosterone. Thus, while the course of sex differentiation may not be susceptible to manipulation by exogenous hormones, the influence of endogenous hormones remains largely unknown (Docker 1992).

The percentage of female southern brook lamprey varied directly with growth under most conditions, the effect being most pronounced when larval density was low and annual mean temperature high. Docker and Beamish did not find a significant relationship between the percentage of females and age group modal length. They did, however, find that fecundity of least brook lamprey increased with the cubic power of total length and also that fecundity, adjusted to a specific length, varied directly with the individual growth rate that characterized each population. It is interesting that the efficiency of fertilization or egg deposition in lamprey is enhanced by the presence of more than a single male (Manion 1968; Hardisty and Potter 1971). Precisely how population size is regulated in populations with a predominance of females is beyond the scope of the present study, but fertilization efficiency may represent one important mechanism.

Male southern brook lamprey were more prevalent under acidic than alkaline conditions, except under conditions of low larval density. A similar effect of pH has been reported for some teleost fishes. A particularly dramatic effect was reported by Heiligenberg (1965) who found the offspring of *Pelvicachromis* to be all male in acidic and all female in alkaline water. Similarly, Rubin (1985) found the offspring from five cichlid species and green swordtail, *Xiphophorus helleri*, to be almost entirely male at low pH and mostly female at neutral pH. The mechanism by which this occurs has not been described, but low pH can influence reproduction in fishes by decreasing the motility of sperm, through damage to genetic material in the developing ova, and by inhibition of hormone production or activity (Fritz 1980; Haines 1981).

Temperature has been implicated in sex differentiation in many lower vertebrates including fish (Conover and Kynard 1981; Conover 1984; Sullivan and Schultz 1986) and reptiles (Yntema 1979; Bull 1980; Deeming and Ferguson 1988). These studies and others suggest that temperature acts early in embryonic or larval development to influence the outcome of primary sex differentiation rather than causing a secondary reversal of gonadal sex. There is some evidence that temperature-dependent sex determination in at least the Atlantic silverside, *Menidia menidia*, is adaptive in that the environment that offspring enter has a gender-dependent effect on fitness (Charnov and Bull 1977; Conover 1984). Sullivan and Schultz (1986) found that in the teleost *Poeciliopsis lucida*, some females are influenced by ambient temperature to produce skewed sex ratios among the offspring, while other females produce approximately equal numbers of male and female progeny. Apparently in *P. lucida*, population sex ratio is influenced by both genetics and environmental quality. In the present study, temperature was not measured during egg incubation or during early larval development. Only the annual mean temperature was estimated which may or may not reflect the thermal regime during early development. It is important to point out that in lamprey, sex is not differentiated for about a year, in contrast with that found for many animals including the Atlantic silverside which completes its life cycle within a single year.

Polygenic sex determination is thought to be the primitive genetic mechanism for sex determination and to have been replaced during the course of evolution first by genic sex determination and then by semichromosomal or chromosomal sex determination (Kirpichnikov 1981; Rice 1986). In the polygenic system the sex of an individual reflects the cumulative effect of environmental quality acting on each of a large number of genes which individually exercise only a small influence on sex. Polygenic sex determination is thought to be rare in extant species (Rice 1986), and sex chromosomes are either absent or in a primitive state of differentiation in fish (Ohno 1967). It is interesting that in the hermaphroditic fish *Rivulus marmoratus*, which lacks sex chromosomes, the gonad develops into a testes rather than ovotestes when ambient temperature is low (Harrington 1971). This suggests that in at least some lower vertebrates, gonadal differentiation is affected by metabolic rate and, by implication, growth.

Environmental sex determination has been proposed as a tactic to optimize fitness in relation to environmental quality (Charnov and Bull 1977). Thus, if sex ratios are adaptive in gonochoristic vertebrates, individuals of large size would be expected to become the sex in which the rewards for being large are greatest or the penalty for being small least (Charnov and Bull 1977; Conover 1984). In populations of both the southern and least brook lamprey, there was seldom a significant difference in length between males and females of the same age. Docker and Beamish (personal observation) suggested that the mechanics of spawning may result in reduced fertilization efficiency when there are large differences in size between individuals. Malmqvist (1983) found that fertilization in *L. planeri* was most successful when length differences between the two sexes were less than 14%.

If sex ratios are indeed adaptive, they may be expected to vary throughout stream length in response to changes in environmental quality. Southern brook lamprey are typically found in relatively small streams in which environmental quality is reasonably homogeneous throughout their entire length. In this context, the present study did not compare sex ratios at specific locations within a stream. However, sex ratio of least brook lamprey was examined at several locations within each of three streams by Docker and Beamish and significant differences were not found.

In summary, the present study provides further evidence of environmental sex determination in the phylogenetically primitive lamprey and attributes the response to several abiotic and biotic factors. It remains unclear whether sex ratios in lamprey are or are not adaptive. Sullivan and Schultz (1986) offered the possibility that sex ratios may simply reflect the outcome of a series of physiological and endocrinological processes without a specific linkage to fitness.

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References

ABAKUMOV, V.A. 1966. Systematics and ecology of the Ukrainian lamprey (*Lampetra mariae* Berg.). Vopr. Ikhtiol. 41: 609–618. (In Russian)

AMERICAN PUBLIC HEALTH ASSOCIATION (APHA), AMERICAN WATER WORKS ASSOCIATION, AND WATER POLLUTION CONTROL FEDERATION. 1989. Standard methods for the examination of water and waste water. 17th ed. Washington, D.C.

APPLEGATE, V.C. 1950. Natural history of the sea lamprey (*Petromyzon marinus*) in Michigan. U.S. Fish Wildl. Serv. Spec. Sci. Rep. Fish. Serv. 55.

ATZ, J.W. 1964. Intersexuality in fishes, p. 145–232. *In* A.J. Marshall and C.N. Armstrong [ed.] Intersexuality in vertebrates, including man. Academic Press, London.

BARDACK, D., AND R. ZANGERL. 1971. Lampreys in the fossil record, p. 67–84. *In* M.W. Hardisty and I.C. Potter [ed.] The biology of lampreys. Vol. 1. Academic Press, London.

BEAMISH, F.W.H. 1982. Biology of the southern brook lamprey, *Ichthyomyzon gagei*. Environ. Biol. Fishes 7: 305–320.

BEAMISH, F.W.H., AND T.E. MEDLAND. 1988. Age determination for lampreys. Trans. Am. Fish. Soc. 117: 63–71.

BEAMISH, F.W.H., AND I.C. POTTER. 1975. The biology of the anadromous sea lamprey (*Petromyzon marinus*) in New Brunswick. J. Zool. Lond. 177: 57–72.

BEAMISH, F.W.H., AND E.J. THOMAS. 1983. Potential and actual fecundity of the "paired" lampreys, *Ichthyomyzon gagei* and *I. castaneus*. Copeia 1983: 367–374.

BEYER, W.H. 1968. CRC handbook of tables for probability and statistics. 2nd ed. Chemical Rubber Company, Cleveland, Ohio. 642 p.

BULL, J.J. 1980. Sex determination in reptiles. Q. Rev. Biol. 55: 3–21.

BULL, J.J., AND R.C. VOGT. 1979. Temperature-dependent sex determination in turtles. Science (Wash., D.C.) 206: 1186–1188.

CHARNOV, E.L., AND J.L. BULL. 1977. When is sex environmentally determined? Nature (Lond.) 266: 828–830.

CONOVER, D.O. 1984. Adaptive significance of temperature-dependent sex determination in a fish. Am. Nat. 123: 297–313.

CONOVER, D.O., AND B.E. KYNARD. 1981. Environmental sex determination: interaction of temperature and genotype in a fish. Science (Wash., D.C.) 213: 577–579.

D'ANCONA, U. 1950. Détermination et différentiation du sexe chez les poissons. Arch. Anat. Microsc. Morphol. Exp. 39: 274–292.

DEAN, B., AND F.B. SUMNER. 1898. Notes on the spawning habits of the brook lamprey (*Petromyzon wilderi*). Trans. N.Y. Acad. Sci. 16: 321–324.

DEEMING, D.C., AND M.W.J. FERGUSON. 1988. Environmental regulation of sex determination in reptiles. Philos. Trans. R. Soc. Lond. B 322: 19–39.

DENDY, J.S., AND D.C. SCOTT. 1953. Distribution, life history and morphological variations of the southern brook lamprey, *Ichthyomyzon gagei*. Copeia 1953: 152–162.

DOCKER, M.F. 1992. Labile sex determination in lampreys: the effect of larval density and sex steroids on gonadal differentiation. Ph.D. thesis, University of Guelph, Guelph, Ont. 269 p.

EPPLE, A., Y. KATZ, AND L. DASHOW. 1982. Stress effects on circulating steroids and catecholamines in fishes. Gen. Comp. Endocrinol. 46: 410.

FERGUSON, M.W.J., AND T. JOANEN. 1982. Temperature of egg incubation determines sex in *Alligator mississippiensis*. Nature (Lond.) 296: 850–853.

FRITZ, E.S. 1980. Potential impacts of low pH on fish and fish populations. U.S. Fish Wildl. Serv. Biol. Serv. Program Natl. Powerplant Team FWS/OBS-80/40.2.

HAINES, T.A. 1981. Acidic precipitation and its consequences for aquatic ecosystems. A review. Trans. Am. Fish. Soc. 110: 669–707.

HARDISTY, M.W. 1954. Sex ratio in spawning populations of *Lampetra planeri*. Nature (Lond.) 173: 874–875.

HARDISTY, M.W. 1960. Sex ratios of ammocoetes. Nature (Lond.) 186: 988–989.

HARDISTY, M.W. 1961. Sex composition of lamprey populations. Nature (Lond.) 191: 116–117.

HARDISTY, M.W. 1965a. Sex differentiation and gonad oogenesis in lampreys. I. The ammocoete gonads of the brook lamprey, *Lampetra planeri*. J. Zool. Lond. 146: 305–345.

HARDISTY, M.W. 1965b. Sex differentiation and gonadogenesis in lampreys. II. The ammocoete gonads of the landlocked sea lamprey, *Petromyzon marinus*. J. Zool. Lond. 146: 346–387.

HARDISTY, M.W. 1979. Biology of the cyclostomes. Chapman and Hall, New York, N.Y. 428 p.

HARDISTY, M.W., AND I.C. POTTER. 1971. The behaviour, ecology and growth of larval lampreys, p. 85–125. *In* M.W. Hardisty and I.C. Potter [ed.] The biology of lampreys. Vol. 1. Academic Press, London.

HARRINGTON, R.W. 1971. How ecological and genetic factors interact to determine when self-fertilizing hermaphrodites of *Rivulus marmoratus* change into functional secondary males, with a reappraisal of the modes of intersexuality among fishes. Copeia 1971: 389–390.

HEILIGENBERG, W. 1965. Color polymorphism in the males of an African cichlid fish. J. Zool. 146: 95–97.

HEINRICH, J.W., J.G. WEISE, AND B.R. SMITH. 1980. Changes in biological characteristics of the sea lamprey (*Petromyzon marinus*) as related to lamprey abundance, prey abundance, and sea lamprey control. Can. J. Fish. Aquat. Sci. 37: 1861–1871.

HUBBS, C.L. 1925. The life cycle and growth of lampreys. Pap. Mich. Acad. Sci. 4: 587–603.

HUBBS, C.L., AND M.B. TRAUTMAN. 1937. A revision of the lamprey genus *Ichthyomyzon*. Univ. Mich. Mus. Zool. Misc. Publ. 35.

HUNTER, G.A., E.M. DONALDSON, J. STOSS, AND I. BAKER. 1983. Production of mono-sex female groups of chinook salmon (*Oncorhynchus tshawytscha*) by the fertilization of normal ova with sperm from sex-reversed females. Aquaculture 33: 355–364.

KIRPICHNIKOV, V.S. 1981. Genetic bases of fish selection. Springer-Verlag, Berlin, Heidelberg, and New York. 410 p.

LEE, D.S., C.R. GILBERT, C.H. HOCUTT, R.E. JENKINS, D.E. MCALLISTER, AND J.R. STAUFFER, JR. 1980 et. seq. Atlas of North American freshwater fishes. North Carolina State Museum of Natural History, Raleigh, N.C. 854 p.

LINDSEY, C.C. 1962. Experimental study of meristic variation in a population of threespine stickleback, *Gasterosteus aculeatus*. Can. J. Zool. 40: 271–312.

MALMQVIST, B. 1983. Breeding behaviour of brook lampreys *Lampetra planeri*: experiments on mate choice. Oikos 41: 43–48.

MANION, P.J. 1968. Production of sea lamprey larvae from nests in two Lake Superior streams. Trans. Am. Fish. Soc. 97: 484–486.

MANION, P.J., AND B.R. SMITH. 1978. Biology of larval and metamorphosing sea lampreys, *Petromyzon marinus*, of the 1960 year class in the Big Garlic River, Michigan, Part II, 1966–72. Great Lakes Fish. Comm. Tech. Rep. 30, Ann Arbor, Mich.

MEDLAND, T.E., AND F.W.H. BEAMISH. 1987. Age validation for the mountain brook lamprey, *Ichthyomyzon greeleyi*. Can. J. Fish. Aquat. Sci. 44: 901–904.

MOHANTY-HEJMADI, P., AND M.T. DIMOND. 1986. Temperature dependent sex determination in the Olive ridley turtle, p. 159–162. *In* H.C. Slavkin [ed.] Progress in developmental biology, Part A. Liss, New York, N.Y.

NATIONAL RESEARCH COUNCIL CANADA (NRC), ASSOCIATE COMMITTEE ON SCIENTIFIC CRITERIA FOR ENVIRONMENTAL QUALITY. 1985. TFM and Bayer 73: lampricides in the aquatic environment. Natl. Res. Counc. Can. Environ. Sect. Pu NRCC No. 22488: 184 p.

OHNO, S. 1967. Sex chromosomes and sex-linked genes. Springer-Verlag, Berlin, Heidelberg, and New York. 192 p.

OKKELBERG, P. 1921. The early history of the germ cells in the brook lamprey, *Entosphenus wilderi*, up to and including the period of sex differentiation. J. Morphol. 35: 1–152.

PIAVIS, W.G. 1971. Embryology, p. 361–400. *In* M.W. Hardisty and I.C. Potter [ed.] The biology of lampreys. Vol. 1. Academic Press, London.

POTTER, I.C. 1980. Ecology of larval and metamorphosing lampreys. Can. J. Fish. Aquat. Sci. 37: 1641–1657.

PURVIS, H.A. 1970. Growth, age at metamorphosis and sex ratio of northern brook lampreys in a tributary of southern Lake Superior. Copeia 1970: 326–332.

PURVIS, H.A. 1979. Variation in growth, age at transformation, and sex ratio of sea lampreys re-established in chemically treated tributaries of the Upper Great Lakes. Great Lakes Fish. Comm. Tech. rep. 35, Ann Arbor, Mich. 36 p.

RANEY, E.C. 1952. A new lamprey, *Ichthyomyzon hubbsi*, from the upper Tennessee River system. Copeia 1952: 95–99.

RICE, W.R. 1986. On the instability of polygenic sex determination: the effect of sex-specific selection. Evolution 40: 633–639.

RICHARDS, C.M., AND G.W. NACE. 1978. Gynogenetic and hormonal sex reversal used in test of the XX–XY hypothesis of sex determination in *Rana pipiens*. Growth 42: 319–331.

RUBIN, D.A. 1985. Effects of pH on sex ratio in cichlids and a poecilliid

(Teleostei). Copeia 1985: 233–235.
SAS. 1989. SAS user's guide: statistics. Vol. 2. SAS Institute Inc., Cary, N.C. 584 p.
SMITH, B.R. 1971. Sea lampreys in the Great Lakes of North America, p. 207–247. *In* M.W. Hardisty and I.C. Potter [ed.] The biology of lampreys. Vol. 1. Academic Press, London.
SMITH, B.R., AND J.J. TIBBLES. 1980. Sea lamprey (*Petromyzon marinus*) in Lakes Huron, Michigan, and Superior: history of invasion and control, 1936–78. Can. J. Fish. Aquat. Sci. 37: 1780–1801.
STIER, K., AND B. KYNARD. 1986. Abundance, size and sex ratio of adult sea-run sea lampreys *Petromyzon marinus* in the Connecticut River USA. U.S. Natl. Mar. Fish. Serv. Fish. Bull. 84: 476–548.
SULLIVAN, J.A., AND R.J. SCHULTZ. 1986. Genetic and environmental basis of variable sex ratios in laboratory strains of *Poeciliopsis lucida*. Evolution 40: 152–158.
VLADYKOV, V.D. 1951. Fecundity of Quebec lampreys. Can. Fish. Cult. 10: 1–14.
WIGLEY, R.L. 1959. Life history of the sea lamprey of Cayuga Lake, New York. U.S. Fish Wildl. Serv. Fish. Bull. 59: 559–617.
WILLEY, R.L. 1971. Microtechniques: a laboratory guide. The Macmillan Co., New York, N.Y. 99 p.
YAMAMOTO, T. 1953. Artificially induced sex reversal in genotypic males of the medaka (*Oryzias latipes*). J. Exp. Zool. 123: 571–594.
YAMAZAKI, F. 1983. Sex control and manipulation in fish. Aquaculture 33: 329–354.
YNTEMA, C.L. 1979. Temperature levels and periods of sex determination during incubation of eggs of *Chelydra serpentina*. J. Morphol. 158: 17–27.
ZANANDREA, G. 1959. *Lampetra fluviatilis* catturate in mare nel Golfo di Gaeta. Pubbl. Staz. Zool. Napoli 31: 265–307.