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Ecology of recruitment in sea lamprey--summary

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B. J. Wicks, B. J. Morrison, L. A. Barker, and F. W. H. Beamish

Department of Zoology
Institute of Ichthyology
University of Guelph
Guelph, Ontario
N1G 2W1

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

Wicks BJ, Barker LA, Morrison BJ, Beamish WH

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BROOK LAMPREY

Addresses:
Barker LA, Univ Guelph, Dept Zool, Inst Ichthyol, Guelph, ON N1G 2W1, Canada.
Univ Guelph, Dept Zool, Inst Ichthyol, Guelph, ON N1G 2W1, Canada.

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Project Completion Report

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

Beverly J. Wicks, Leslie A. Barker, Bruce J. Morrison, and William H. Beamish
Department of Zoology, Institute of Ichthyology,
University of Guelph, Guelph, Ontario, Canada, N1G 2W1

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Abstract.- The observation of atypical gonads in land-locked sea lamprey larvae from several streams tributary to the Great Lakes led to this descriptive study on typical male and female gonads, as well as atypical gonads. Typical male and female gonads examined in the present study have similar characteristics to those observed in the past; however, larvae with atypical gonads have some characteristics of both male and female larvae. Many of the atypical larvae had an unusual number of germ cells, or many atretic oocytes. Based on quantitative evaluation of several gonadal characteristics of male, female and atypical larvae, it was possible to verify atypical status of 88 % of the larvae which had been qualitatively assigned the atypical sex. The slowing of gonadogenesis, or the diversion of energy for somatic development are possible causes of the atypical gonads observed in this study.

Introduction

Sea lampreys, *Petromyzon marinus*, have an indirect life cycle (Balon 1990) in which the larvae inhabit the soft sediment of freshwater streams generally for three to six years (Applegate 1950; Wigley 1959). During the subsequent juvenile period, sea lampreys are active predators of fishes. In the Great Lakes basin, sea lamprey abundance is controlled in part by the regular application of a chemical, 3-trifluoromethyl 4-nitrophenol (TFM), to streams inhabited by larvae (Smith 1971; Smith and Tibbles 1980).

Gonadogenesis in land-locked sea lamprey begins when larvae reach total lengths of 40 - 65 mm, which usually occurs at 1 - 2 years of age (Hardisty 1965, 1969; Lewis and McMillan 1965; Docker 1992). Typically, sex differentiation is complete when larvae reach total lengths of 90 - 100 mm, which usually occurs at 2 - 3 years of age (Hardisty 1965, 1969; Lewis and McMillan 1965; Docker 1992). Sex ratios have changed dramatically with sea lamprey abundance, particularly since the commencement of chemical control. During high sea lamprey abundance following their colonization of the Upper Great Lakes, adult male lamprey comprised as much as 70% of the population (Heinrich et al.,1980). Larval populations of tributaries to Lake Superior and Lake Huron shifted from 30 - 35% females in 1958 to as much as 84% females in 1965, with the reduction in lamprey numbers (Purvis, unpubl.).

Recent observations of sea lamprey larvae from three streams tributary to the Great Lakes indicated marked differences in the morphology and cytology of gonads from previous descriptions (Hardisty 1965,1969; Lewis and McMillan 1965; Docker 1992). This paper describes these variations and suggests causal mechanisms.

Material and Methods

Gonadal characteristics were examined in sea lamprey larvae collected from Cobourg Brook (43° 58' latitude and 78° 10' longitude), Farewell Creek (43° 52 ' latitude and 78° 49' longitude), both tributaries to the north shore of Lake Ontario and Little Gravel River (48° 18' latitude and 88° 43' longitude) a tributary to Lake Superior. Larvae were electrofished from Cobourg Brook on May 31 and June 18 1996, from Farewell Creek on June 10 and September 20 1995 and from Little Gravel river on July 12 1995. All larvae were killed by an overdose of tricaine methanesulfate (MS 222), measured for total length (±1 mm) and preserved in 5% formalin.

Gonad morphology and cytology in sea lamprey larvae greater than 90 mm total length from Cobourg Brook were examined from histological transverse sections *in toto* at both 4 and 10 microns. Tissue blocks, approximately 3-5 mm thick were taken from the mid-section of each larvae for gonad examination. In a previous study (L. Barker, B. Morrison, B. Wicks, F. Beamish, unpubl.), gonads of sea lamprey larvae were sectioned at five locations equally distributed throughout the length of the gonad. Number of germ cells and oocytes did not differ significantly throughout the anterior half of the gonad, aside from the first few millimetres. In the posterior half of the gonad, absolute numbers of germ cells and oocytes declined with gonad area, but relative numbers were consistent with those in the anterior half of the gonad. Oocyte and germ cell size did not significantly differ throughout the entire length of gonad.

In the present study, each tissue block taken from the mid-region of the larvae was dehydrated in successively increasing concentrations of ethanol (50-100%), cleared in xylene and embedded in paraffin. Approximately 10 sections were taken, which were at least 10 sections apart. Sectioned tissue was stained with Harris' haematoxylin and counterstained with eosin or acid washed with 1% HCl in 70% ethanol (Humason, 1979).

Criteria for sexing sea lamprey larvae for this investigation were based on a combination of previously reported criteria described for larvae > 90 mm in total length (Hardisty 1965, Lewis and McMillan 1965, Docker 1992). Females have a single horseshoe-shaped ovary, consisting of finger-like lobes that contain two rows of oocytes (Docker 1992). Early stage oocytes can be distinguished from other cells, including primordial germ cells, by greater amounts of cytoplasm resulting in larger cell size, somatic elements forming follicle cells around the oocyte (Hardisty 1965; Lewis and McMillan 1965) and a large empty space within the oocyte, described by Hardisty (1965) as an accessory nuclear vesicle. Second stage oocytes are easily identified by a dense basophilic cytoplasm and an average maximum oocyte diameter of > 60 um (Hardisty 1965). In female larvae, as many as 250 oocytes may be present in a single transverse section but usually they range from 80 - 200 per transverse section (Docker 1992). Counts of undifferentiated germ cells are usually < 5 per section in female larvae. The smaller gonad of the male lamprey has a more angular morphology than the female with shallow clefts in the margin (Docker 1992). Male gonads have a large amount of stromal tissue and isolated or nested germ cells with 6 - 20 germ cells per cell nest (Hardisty 1965). Oocytes may be present but are on average < 18 um maximum diameter and rarely exceed six per transverse section (Docker 1992).

Gonad area ($mm^2 \pm 0.001$ mm^2) and perimeter (mm ± 0.001 mm) excluding the hilar region, the connective and vascular tissue attaching the gonad to the kidney tissue, were measured using a computerized digitizer from each transverse section. Oocyte diameters were measured using an ocular micrometer. Oocyte diameter for females was taken as the mean of the 10 largest diameters in each section. In males, all oocytes identified in each transverse section were measured and a mean oocyte diameter calculated. When oocytes were at asynchronous stages of development, all oocytes were measured and were represented by the mean. The shape of the gonad was represented by perimeter^2:area (P^2/A) (Docker 1992).

Sex was assigned qualitatively based on descriptions in the literature. In addition, germ cell number, oocyte number, oocyte diameter, number of atretic oocytes, and gonad area, perimeter, and shape were measured to assess the ability to correlate the assigned sex with the measurements taken. These gonadal characteristics were quantified extensively in larvae from Cobourg Brook (N= 91); in Farewell Creek and Little Gravel River, the gonadal characteristics were quantified in only 5 and 3 atypical larvae respectively. Data from Cobourg Brook was analysed using a multi-variate analysis of variance (MANOVA, SAS, 1985) to determine generally which gonadal characteristics could be used to discriminate between male, female and atypical larvae. Normality was tested by examination of residuals (Johnson and Wichern, 1982).

The tolerance limits (Odeh and Owen, 1980) for each suitable gonadal characteristic, as determined above, were calculated for male and female larvae from Cobourg Brook, respectively over four total length intervals (90 - 105, 106 - 120, 121 -135 and greater than 136 mm). The larvae were classed into total length intervals because as total length increases, there are corresponding changes in germ cell number, oocyte number, oocyte diameter, gonad perimeter and area (Hardisty, 1965).

We then compared the values for each gonadal characteristic measured on individual atypical larvae from Cobourg Brook, Farewell Creek and Little Gravel River to the tolerance limits calculated for typical male and female larvae in Cobourg Brook. Confirmation of atypical status for individual larvae was accepted when one or more of the gonadal characteristics was outside the tolerance limits calculated for typical males and females. In addition, confirmation of atypical status for individual larvae was accepted when one or more of the gonadal characteristics was in the tolerance limit for typical females, while other gonadal characteristics were in the tolerance limits for typical males. If however, all gonadal characteristics were in the tolerance limit calculated for either typical males or females, atypical status was rejected and the larvae were re-classified as the respective sex.

Results

Many of the larvae examined from Cobourg Brook, Farewell Creek and Little Gravel River were easily distinguished as male (Figure 1A) or female (Figure 1B) based on established criteria (Hardisty 1965, Lewis and McMillan 1965, Docker 1992). However, sex of some larvae could not be assigned based on these criteria. Of the 91 larvae examined from Cobourg Brook, 22 were clearly males (90 - 157 mm) and 22 were clearly females (115 - 155 mm). Gonads in all of the remaining larvae (90 - 154 mm) displayed morphological and cytological characteristics of both males and females to varying degrees and were termed atypical larvae (Figure 2A, 2B and 2C). Larvae examined from Farewell Creek (N = 179) and Little Gravel River (N= 93) had similar characteristics to that of larvae from Cobourg Brook. Thirty-three percent of larvae examined from Farewell Creek and 80% from Little Gravel River exhibited atypical gonads. Testes of the typical male larvae had an angular shape with shallow clefts (Figure 1A). The gonad area and perimeter were small relative to females, ranging from 0.014 - 0.077 mm^2 and 0.88 - 2.35 mm, respectively (Table 1). Testes contained isolated or clusters of germ cells (Figure 1A) which ranged in number from 4 - 598 per transverse section. Few oocytes were present and their maximum diameter was always less than 22 μm (Table 1). Ovaries of typical females were horseshoe shaped with finger-like projections (Figure 1B), resulting in a larger gonad area and perimeter than those observed in males (Table 1). Ovary shape was less variable than for males and ranged from 106 - 149. Oocytes had well defined margins and basophilic cytoplasm (Figure 1B). In typical females, ovaries contained up to 190 oocytes per transverse section and their maximum diameters ranged from 46 - 79 μm (Table 1). None of the ovaries examined contained atretic oocytes. Some of the ovaries examined contained several clusters of germ cells resulting in up to 160 germ cells per transverse section; while others were without germ cells.

Gonadal characteristics of some atypical larvae were similar to those of typical males (Figure 2A). These larvae had a small gonad area and perimeter, 0.032 - 0.131 mm^2 and 1.65 - 3.42 mm respectively, similar to the range found for typical males. However the number of germ cells tended to be much higher than in typical males with numbers in some in excess of 1300. In addition, some of these larvae had up to 30 oocytes per transverse section, much higher than in typical males (Table 1). Other atypical larvae contained horseshoe shaped gonads with finger-like projections, much like a typical ovary (Figure 2B). The gonad area and perimeter were 0.116 - 0.715 mm^2 and 2.22 - 9.30 mm respectively, similar to those observed in typical females. However, these gonads generally contained fewer than 30 oocytes per transverse section and as many as 85 atretic oocytes. In addition, many of these atypical larvae had germ cell numbers that were much greater than in typical females, in some in excess of 700 (Figure 2B). Other atypical larvae contained gonads with an angular shape, much like a typical male; however, the gonads also had large finger-like projections, resulting in a large gonad area and perimeter (Figure 2C).

Discussion

Atypical gonadal development was commonly observed among land-locked sea lamprey larvae of a total length greater than that normally associated with sexual differentiation. Some larvae with atypical gonads displayed characteristics found in testes, while others displayed ovarian characteristics. However, larvae with atypical gonads were statistically distinct from both male or female larvae on the basis of germ cell number, oocyte number, oocyte diameter, number of atretic oocytes, gonad perimeter, gonad area and gonad shape.

In an earlier account of ovarian development in land-locked sea lamprey larvae, Lewis and McMillan (1965) described the occasional individual with unusual gonadal characteristics. They did not provide greater detail, but used the term "hermaphroditic larvae" to describe the condition of the gonads. Fukayama and Takahashi (1982), also observed unusual gonads in the parasitic Japanese river lamprey, *Lampetra japonica*. Sexual differentiation of male Japanese river lampreys occurred in one of two ways. A testis either developed directly from an undifferentiated gonad, or a gonad first differentiated as an ovary, and then through a transitional stage before ultimately becoming a testis. The gonads of some atypical sea lamprey larvae in the present study resembled those described by Fukayama and Takahashi (1982).

A description of gonad status based on extensive histological examination on large numbers of sea lamprey larvae was undertaken for Cobourg Brook only. However, in an additional 13 streams, large numbers of sea lamprey larvae (160 ± 76, $\bar{x} \pm$ SD) were subjected to less extensive histological examination of their gonads (B. J. Wicks, B. J. Morrison, L. A. Barker and F. W. H. Beamish, unpubl.). The percent atypical larvae varied from 3 to 100%, with a mean ($\pm$ SD) of 46 ± 32. This contrasts with Fukayama and Takahashi (1982), who observed that about 10 % of male Japanese river lampreys exhibited unusual gonadal characteristics.

Sea lamprey larvae have a long period of sexual indeterminacy and germ cells have the capability to develop into either male or female sex cells (Hardisty, 1965). Many of the atypical larvae examined in this study had an unusual number of germ cells per transverse section (up to 1300), which may extend the period of sexual differentiation, during which it may be labile and influenced by abiotic and biotic factors (Smith, 1971; Beamish, 1993; Docker and Beamish, 1994). Generally, sea lampreys commence metamorphosis after a larval period of 4 - 6 years (Applegate, 1950). However, streams tributary to the Great Lakes containing sea lampreys are treated with lampricide at intervals of approximately three years. This imposes selection pressure to complete the larval period and initiate metamorphosis within the treatment cycle. One strategy may be to allocate a disproportionate amount of energy to somatic growth in order to shorten the larval period, at the expense of gonadal development. Gonadal development would presumably resume during the juvenile period which follows the non-trophic phase of metamorphosis.

The current study describes sea lamprey larvae with gonad phenotypes dissimilar to the definitive male and female larval gonads previously observed. The phenotypes are not proposed as new, but previously were rarely seen. Slowing of gonadogenesis may be responsible for changes to the phenotypic frequencies. The fate of atypical larvae post-metamorphosis is also of interest both biologically and for the predictive needs of management.

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Table 1. - Ranges of gonadal characteristics for male, female and atypical sea lamprey larvae, *Petromyzon marinus*, examined from Cobourg Brook, Ontario. Values given are means of measurements made from 10 transverse sections per larvae.

Gonadal Characteristics	Sex		
	Male (N = 22)	Female (N = 22)	Atypical (N = 47)
Germ cell number	4 - 598	0 -159	4 - 1372
Oocyte number	0 - 8	75 - 190	0 - 167
diameter (μm)	13 - 22	46 - 77	16 - 79
Number of atretic oocytes	0	0	0 - 84
Gonad area (mm^2)	0.014 - 0.077	0.216 - 0.823	0.032 - 0.715
perimeter (mm)	0.88 - 2.35	4.75 - 11.02	1.21 - 9.30
shape (P^2/A)	56 - 164	106 - 149	45 - 140

Table 2. Tolerance limits for each gonadal characteristic measured on male and female larval sea lampreys, *Petromyzon marinus*, from Cobourg Brook. The tolerance limits are given for germ cell number, oocyte number, oocyte diameter, number of atretic oocytes, gonad area, gonad perimeter and gonad shape for three total length ranges; 106 - 120 mm, 121 - 135 mm and greater than 136 mm.

Gonadal characteristic	Larval length range (mm)	Male tolerance limit	Female tolerance limit
Germ cell number	106 - 120	-418, 1157	-81, 193
	121 - 135	-81, 700	-413, 658
	> 136	-413, 658	-22, 36
Oocyte number	106 - 120	0	47, 172
	121 - 135	-1.2, 1.9	64, 216
	> 136	-8.8, 10.2	30, 238
diameter (μm)	106 - 120	-	42.9, 53.6
	121 - 135	14.7	38.7, 73.5
	> 136	11.9, 17.7	46.1, 93.6
Number of atretic oocytes	106 - 120	0	0
	121 - 135	0	0
	> 136	0	0
Gonad area (mm^2)	106 - 120	0.013, 0.067	0.123, 0.449
	121 - 135	0.036, 0.039	0.064, 0.752
	> 136	-0.033, 0.098	0.066, 1.120
perimeter (mm)	106 - 120	0.50, 2.40	3.24, 8.61
	121 - 135	0.20, 3.22	2.90, 10.83
	> 136	0.11, 3.03	2.15, 14.79
shape (P^2/A)	106 - 120	19, 80	64, 161
	121 - 135	-87, 266	64, 173
	> 136	-4, 152	43, 205

Legend for Figures

Figure 1. Transverse sections from the mid-region of typical sea lamprey larvae, *Petromyzon marinus*. (A) A gonad from a typical 138 mm male larvae; there are small clusters of germ cells in small, angular shaped testis (t) (100X). (B) A gonad from a typical 123 mm female larvae; oocytes are all basophilic and there is no evidence of atresia; the large ovary (ov) is horseshoe shaped with finger-like extensions (100X).

Figure 2. Transverse sections from the mid-region of atypical sea lamprey larvae, *Petromyzon marinus*. (A) A gonad from a 125 mm atypical larvae with some male characteristics; the large gonad has several large germ cell clusters (g) (100X). (B) A gonad from a 116 mm atypical larvae with some female characteristics; the large gonad is horseshoe shaped and shows the large germ cell clusters (g) and very few oocytes (100X). (C) A gonad from a 146 mm atypical larvae with characteristics of both male and female larvae; the large gonad is angular shaped with lobes and many germ cells clusters (g) are present; several first stage oocytes (o) are present (100X).

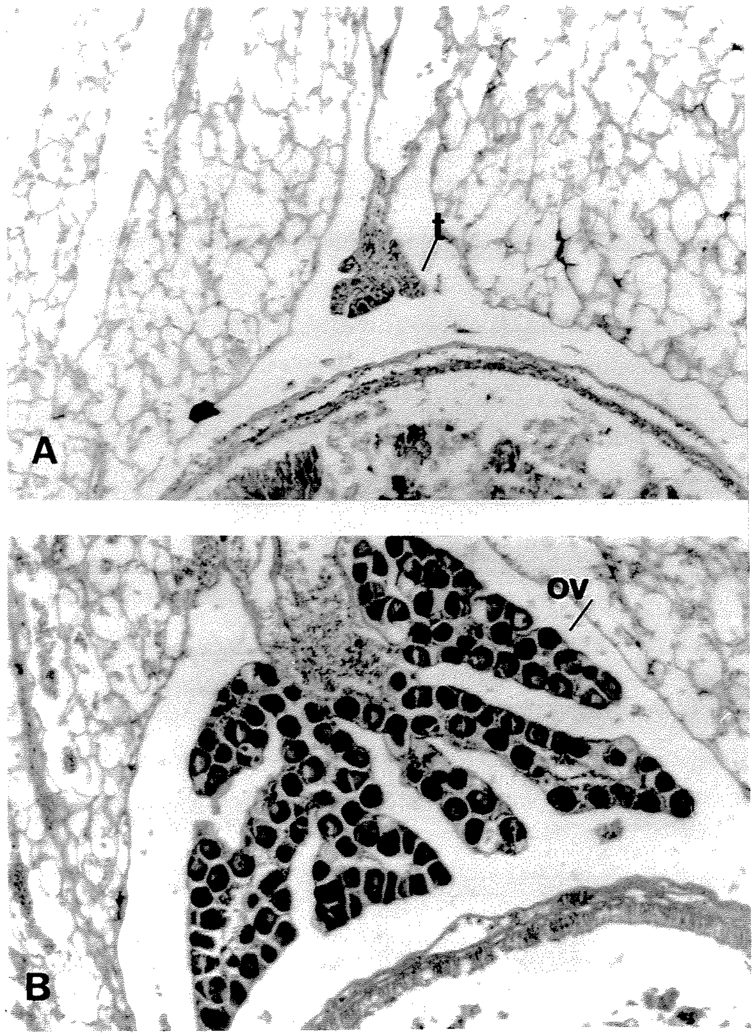

Figure 1

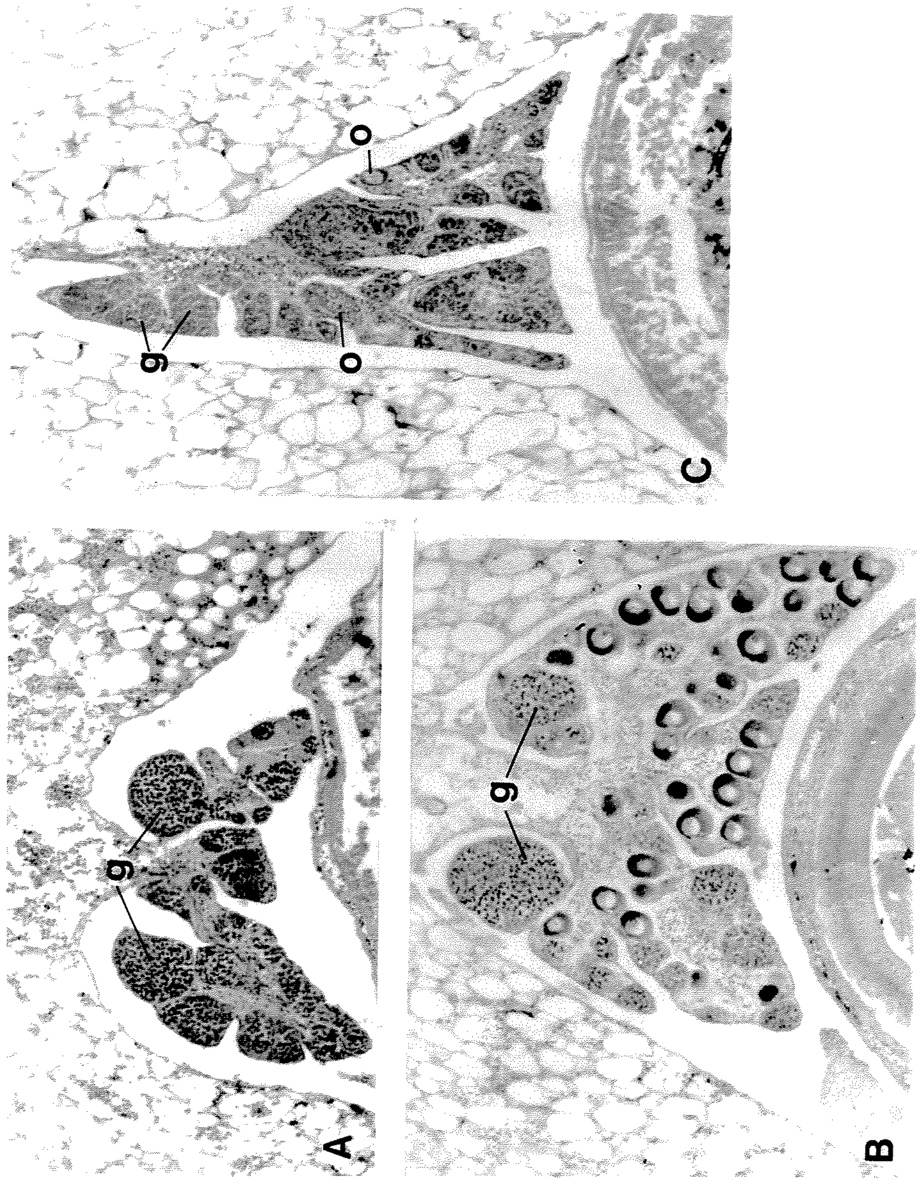

Figure 2.