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March 1998

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COPEIA
(4) 1070-1075 DEC 30 1998

Document type: Article **Language:** English **Cited References:** 16 **Times Cited:** 4

KeyWords Plus:
ICHTHYOMYZON-GAGEI, BROOK LAMPREY

Addresses:
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Publisher:
AMER SOC ICHTHYOLOGISTS HERPETOLOGISTS, LAWRENCE

IDS Number:
184VL

ISSN:
0045-8511

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GREAT LAKES FISHERY COMMISSION

Project Completion Report

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

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March, 1998

accepted for publication in *Copeia*

Introduction.---Lampreys produce a fixed number of oocytes early in larval life, which represents their total reproductive potential, or potential fecundity (Beamish and Thomas, 1983). In the landlocked sea lamprey of the Great Lakes, considerable oocyte atresia is thought to occur in post-larval stages (Hardisty, 1965b; 1969) so that actual fecundity may be appreciably less than potential fecundity (Applegate, 1950; Vladykov, 1951; Hardisty, 1965b). In the anadromous sea lamprey, the actual fecundity of adults and potential fecundity of larvae are similar (Hardisty, 1964). The much lower actual fecundity of the landlocked sea lamprey is thought to be directly related to the smaller adult body size (Hardisty, 1964; Beamish and Thomas, 1983).

Sex differentiation in landlocked sea lamprey is typically complete when larvae reach total lengths of approximately 100 mm (Hardisty, 1965a, 1965b, 1969; Docker, 1992). Females are characterized by a large horseshoe-shaped ovary with finger-like lobes, each containing two rows of oocytes greater than 60 μm in diameter (Hardisty, 1965b; Docker, 1992). Stromal tissue is scarce between the germinal elements, and there are few undifferentiated germ cells (Docker, 1992). Undifferentiated, or primordial germ cells develop into oocytes through two growth phases. During the first growth phase, occurring in larvae 80 to 100 mm, cells increase synchronously in size (12 μm to 40 μm diameter) and are called first stage oocytes (Hardisty, 1971). A second growth phase follows, with oocytes synchronously increasing in diameter to 80 μm. These 'second stage oocytes' are typically found in larvae greater than 110 mm total length (Hardisty, 1971). Males have an angular-shaped testis without lobes (Docker, 1992). Oocytes may be present but are few in number and usually less than 18 μm in diameter (Docker, 1992). Stromal tissue is abundant and

clusters of germ cells are present (Hardisty, 1965b).

Recent observations (B. Wicks, L. Barker, B. Morrison, F. W. Beamish, unpubl.) on landlocked sea lamprey larvae from the Great Lakes basin have revealed highly atypical patterns of gonadal development. Gonads of larvae with atypical gonads exhibit varying degrees of male and female characteristics. For example, gonad morphology may be similar to a female; however, primordial germ cells may be abundant. Oocytes may be asynchronous in their development, such that both first and second stage oocytes are present, or a high proportion of atretic oocytes may be present. The present study was undertaken to compare potential fecundity of typical and atypical larvae collected from streams tributary to the Great Lakes. The present study also compared current estimates of potential fecundity with those made several decades earlier (Hardisty, 1964) before larval abundance was regularly reduced in streams by chemical treatment.

Materials and Methods.---In September, 1995 sea lamprey larvae were captured by electro-fishing from Cannon Creek, a tributary to the north shore of Lake Huron and from Lynde Creek, a tributary to Lake Ontario. In June, 1996 sea lamprey larvae were captured by electro-fishing from Cobourg Brook, a tributary to Lake Ontario. In addition, sea lamprey larvae were captured by electro-fishing from Gordon's Creek, a tributary to Lake Huron, in June, 1995 and October, 1996. Larvae were killed by an overdose of MS-222 and total length was measured ($\pm$1 mm) prior to preservation in 5% formalin.

Transverse sections from the mid-region of the body were examined histologically and categorized as males, females or atypical larvae based on criteria in Hardisty (1965b), Lewis

and McMillan (1965) and B. Wicks, L. Barker, B. Morrison, F.W. Beamish (unpubl.). A sub-sample of these larvae was examined for potential fecundity. The trunk region from the posterior margin of the seventh branchiopore to the cloaca was cut into 3 mm ($\pm$0.5 mm) sections and, after fixation, embedded in paraffin wax. Serial transverse sections were cut through the entire length of gonad at either 7 μm (atypical larvae) or 17 μm (females). Tissue sections were stained with haematoxylin and either counterstained with eosin or acid-washed in 1% hydrochloric acid solution in 70% ethyl alcohol (Humason, 1979).

Primordial germ cells, oocytes and spermatocytes are all described as germ cells (Hardisty, 1964); however, in the present study, the term 'germ cells' refers only to primordial germ cells. In addition, first and second stage oocytes are categorized together, unless otherwise stated.

Potential fecundity was determined by counting second stage oocytes in one of every nine transverse sections. Total number of oocytes was calculated using the method in Beamish and Thomas (1983). In a preliminary study of one typical female lamprey in which 3,500 transverse sections were examined, oocytes were distributed evenly throughout the anterior half of the ovary, except for the first few millimetres. In the posterior half of the ovary, oocyte numbers slowly declined to zero just anterior to the cloaca. On the basis of this distribution, fecundity for other larvae was estimated from oocyte counts from a sub-sample of five, 3-mm blocks along the length of the ovary, which represented approximately 25% of the total ovary length. One block each was taken at the anterior and posterior extremes of the ovary, with the remaining three blocks taken at equal intervals between the anterior and posterior portions of the gonad. The numbers of atretic oocytes, those that are degenerating or

being resorbed, were also counted.

The number of germ cells was estimated for typical and atypical females by examining all transverse sections from the five, 3-mm blocks and extrapolating over the ovary length. Oocyte diameters were measured using an ocular micrometer. Maximum oocyte diameter was calculated as the mean of the 10 largest diameters in one cross-section taken from the mid-section of the gonad. Cross-sectional area ($mm^2 \pm 1\ mm^2$) of each gonad was measured with a digitizer at the mid-section of the gonad.

Slopes and intercepts of the regressions relating total length of lamprey to maximum oocyte diameter, cross-sectional area and gonad length were calculated and examined for statistical significance by using simple linear regressions (Wilkinson, 1990). Tests of differences between germ cell number and oocyte number in typical and atypical larvae were calculated and examined for statistical significance with t-tests (Wilkinson, 1990). In all tests, the level of significance was considered at $P < 0.05$.

Results.---Relative abundance of larvae with atypical gonads varied from 9 to 82 % of those examined from the four streams (Table 1). Data from Gordon's Creek suggested that there may be annual variation in the proportion of larvae with atypical gonads within individual streams, as the percentage of atypical larvae varied from 82 to 14% in 1995 and 1996, respectively.

Gonads of the typical female larvae examined (n = 8) were horseshoe shaped with lobes each containing two rows of second stage oocytes and small clusters of germ cells (Table 2, Figure 1A). Potential fecundity of typical female larvae ranged from $33 \cdot 10^3$ to $129 \cdot 10^3$. Of

this total, germ cell numbers ranged from $0.5 \cdot 10^3$ to $80 \cdot 10^3$ and oocytes from $19 \cdot 10^3$ to $65 \cdot 10^3$. Atresia of oocytes was not observed in any of the typical ovaries. Maximum oocyte diameter (range, 56 -- 88 μm) of typical female larvae increased with total length, (115 -- 165 mm) and is described by the regression,

$$D = -20.49 + 0.65L \quad (N = 8, r = 0.96)$$

where D is maximum oocyte diameter in μm and L is total length in mm. Cross-sectional area (0.32 -- 1.17 mm^2) also increased with total length of larvae and is described by the regression,

$$A = -1.30 + 0.02L \quad (N = 8, r = 0.90)$$

where A is cross-sectional area of the gonad in mm^2. Similarly, gonad length (50 -- 64 mm) of the typical female larvae increased with total length and is described by the regression,

$$G = -9.11 + 2.54L \quad (N = 8, r = 0.94)$$

where G is gonad length in mm.

The 15 atypical larvae that we examined displayed a variety of gonadal characteristics (Figures 1B to 1F). For convenience, larvae were placed into four groups (Types 1 -- 4) based on gonad area, oocyte diameter, and gonad morphology and cell composition (Tables 2 and 3). Gonad shape varied from angular, without lobes (Type 1), to horseshoe with lobes (Type 3, 4), or horseshoe without lobes (Type 2). Gonad area varied widely from $4 \cdot 10^{-2}$ (Type 1) to $72 \cdot 10^{-2}$ mm^2 (Type 3). Germ cells ranged in number from 0 (Type 1, 3) to $5.3 \cdot 10^5$ (Type 2) for atypical larvae compared with $1.3 \cdot 10^3$ to $80 \cdot 10^3$ for typical larvae; however, differences in germ cells between typical and atypical larvae were not significant ($t = 1.06$, $df = 21$). Some atypical larvae had gonads containing many atretic oocytes (Type 2, 4), while

others showed no evidence of atresia (Type 1, 3). Oocyte diameters of atypical larvae ranged from 15 (Type 1) to 58 μm (Type 3), compared with 56 to 88 μm for typical larvae. Oocytes ranged from $8 \cdot 10^3$ to $51 \cdot 10^3$ (Type 2) in atypical larvae and were significantly less numerous than in typical gonads ($t = -2.21$, $df = 21$). Gonad length of the atypical larvae ranged from 44 (Type 3) to 58 mm (Type 4) and was significantly shorter than typical larvae ($t = -3.41$, $df = 21$)

Potential fecundity of the atypical larvae varied widely from $12 \cdot 10^3$ to $53 \cdot 10^5$. In type 2 and 4 atypical larvae (Table 3), potential fecundity was much higher than found for typical larvae, due to large numbers of germ cells, up to $53 \cdot 10^5$. Potential fecundity of Type 1 and 3 larvae was within the range found for typical larvae (Table 3); but in these instances the gonads displayed unusual characteristics. For example, some Type 1 larvae had a small, angular-shaped gonad lacking lobes, and contained only first stage oocytes. Type 3 atypical larvae had a typical horseshoe-shaped gonad with lobes (Table 2); however, a large amount of stromal tissue was present in the gonad and some lobes did not contain any oocytes.

In atypical larvae, there was no significant correlation between maximum oocyte diameter and total length of larvae ($N = 15$, $r = 0.18$), nor between gonad length and total length of larvae ($N = 15$, $r = 0.60$). Similarly, the correlation between cross-sectional area of the gonad and total length of larvae was not significant ($N = 15$, $r = 0.16$).

Discussion.--- Potential fecundity estimates made in the present study for typical larvae were lower than those made earlier by Hardisty (1964). He estimated potential fecundity for six larvae (103 -- 144 mm total length) from the Ocqueoc River, a tributary to Lake Huron, to

be 110,000 -- 165,000. Our estimates of potential fecundity for typical sea lamprey larvae are however, within the range reported for adult fecundities of 24,021 -- 162,439 (Applegate, 1950; Vladykov, 1951; T. Morse, unpubl.). Hardisty (1965b, 1969) attributed the discrepancy in oocyte number between the larval and adult period to atresia. The present study provided no evidence of atresia in typical larvae, suggesting that if atresia does occur, it likely takes place among germ cells early in their development, before larvae reach the size examined this study. Atresia in post-larval stages to the extent proposed by Hardisty (1965b, 1969) likely does not occur. It is more likely that the potential fecundities reported by Hardisty were over-estimates. Our estimates of oocyte number per transverse section and maximum oocyte diameter were similar to those of Hardisty (1964); however, Hardisty did not consider that an oocyte would be sectioned and counted more than once, which would lead to over-estimation of potential fecundity (Hardisty, personal communication).

Potential fecundity varied widely among atypical larvae. In some larvae with atypical gonads, germ cell numbers were unusually high. Hardisty (1964), Lewis and McMillan (1965) and Docker (1992) did not observe high numbers of germ cells in large landlocked sea lamprey larvae collected from tributaries to Lake Huron, Lake Erie and Lake Champlain, respectively (sample sizes ranged from 6 to 335 larvae). Fukayama and Takahashi (1982) however, observed high numbers of germ cells in the Japanese river lamprey, *Lampetra japonica*. Germ cells ranged from $1.2 \cdot 10^5$ to $3.98 \cdot 10^6$ in larvae 60 to 120 mm total length. In the Japanese river lamprey, differentiation of ovaries occurs at lengths of 70 to 90 mm, with synchronous development of germ cells into oocytes. However, testicular differentiation occurs either directly from the undifferentiated gonad at lengths up to 120 mm, or by first differentiating as

an ovary, then through a transitional stage to ultimately become a testis. Hardisty (1965b) and Lewis and McMillan (1965) reported that the gonads of sea lamprey differentiated directly into ovaries or testes. In contrast, in our study it appears that some of the atypical larvae examined may be males, with the gonad differentiating through an ovarian, then transitional, gonad before the testis develops.

In other larvae with atypical gonads, potential fecundity was within the range found for typical larvae; however, the oocytes were comprised of only first stage oocytes. Hardisty (1965b, 1971) did not observe first stage oocytes in female larvae after they reached 90 -- 100 mm total length. Perhaps these atypical larvae are indeed females, with the gonad initially differentiating as a testis, followed by ovarian differentiation. These unusual characteristics are further emphasized by the poor correlations of oocyte diameter, gonad area and gonad length with total length, whereas, typical larvae showed strong correlations. Although our sample sizes were small, there was no indication that certain types of atypical gonads occurred only certain streams.

Overall, our data show that sea lamprey larvae with atypical gonads displayed a slowing of gonadogenesis, as evidenced by the persistence of germ cells into larger, older larvae. This may indicate they are diverting a disproportionate amount of energy to somatic growth at the expense of gonadal development, thereby allowing them to shorten the larval period. In this regard larval growth rates appear to have accelerated since the 1960's before streams were regularly treated with the lampricide, 3-trifluoromethyl-4-nitrophenol (TFM). Prior to the regular 3 -- 4 year application of lampricide to streams, the larval period was completed in 4 -- 7 years (Applegate, 1950; Stauffer, 1962). Currently, the larval period is

frequently completed in 3 -- 4 years (B. Morrison, L. Barker, F.W. Beamish, unpubl.). Gonadal development presumably resumes during the juvenile period which follows the larval period and the non-trophic interval of metamorphosis.

Sea lampreys have a long period of sexual indeterminacy, (Hardisty, 1965a, 1965b). The persistence of germ cells into larger and older larvae may extend the period of sex indeterminacy. There is evidence to suggest that sex differentiation in lampreys is labile and subject to influence by biotic as well as abiotic factors (Smith, 1971; Purvis, unpubl; Beamish, 1993; Docker and Beamish, 1994). With the reduction of larval density due to the chemical control program female sea lampreys clearly became the dominant sex. Similarly, the proportion of female least brook lamprey, *Lampetra aepyptera*, and southern brook lamprey, *Ichthyomyzon gagei*, was inversely related to density (Beamish, 1993; Docker and Beamish, 1994). The extended period of sex determinacy may allow larvae to compensate to the dramatic changes in abundance by altering their sex.

Acknowledgements.---We are grateful for the financial support provided by the Great Lakes Fishery Commission and the Environmental Youth Corps (Ministry of Natural Resources). Technical assistance provided by D. Lynn was greatly appreciated. Many thanks to T. Frezza and J. Keene for their assistance with counting. Collecting permits were kindly provided by the Ontario Ministry of Natural Resources. This study was carried out under an Animal Utilization Protocol approved by the Animal Care Committee of the University of Guelph.

Literature Cited.

Applegate, V. C. 1950. Natural history of the sea lamprey (*Petromyzon marinus*) in Michigan. U.S. Dep. Interior Fish Wild. Serv. Spec. Sci. Rep. Fish. No. 55.

Beamish, F. W. H. 1993. Environmental sex determination in southern brook lamprey, *Ichthyomyzon gagei*. Can. J. Fish. Aquat. Sci. 50:1299--1307.

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.

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. Natl. Libr. Can.

Docker, M. F. and F. W. H. Beamish. 1994. Age, growth, and sex ratio among populations of least brook lamprey, *Lampetra aepyptera*, larvae: an argument for environmental sex determination. Environ. Biol. Fish. 41:191--205.

Fukayama, S. and H. Takahashi. 1982. Sex differentiation and development of the gonad in the Japanese River Lamprey, *Lampetra japonica*. Bull. Fac. Fish. 33(4): 206--216.

Hardisty, M. W. 1964. The fecundity of lampreys. Arch. Hydrobiol. 60:340--357.

Hardisty, M. W. 1965a. Sex differentiation and gonadogenesis 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. 1969. A comparison of gonadal development in the ammocoetes of the

landlocked and anadromous forms of the sea lamprey, *Petromyzon marinus* L. J. Fish. Biol. 1:153--166.

Hardisty, M. W. 1971. gonadogenesis, Sex Differentiation and Gametogenesis. p. 295--359. *In*: The Biology of Lampreys, Volume 1. M. W. Hardisty and I. C. Potter (eds.). Academic Press, London.

Humason, G. L. 1979. Animal tissue techniques. W. H. Freeman and Company, San Francisco.

Lewis, J. C. and D. B. McMillan. 1965. The development of the ovary of the sea lamprey (*Petromyzon marinus* L.). J. Morph. 117:425--466.

Smith, B. R. 1971. Sea lampreys in the Great Lakes of North America. p. 207--247. *In*: The Biology of Lampreys, Volume 1. M. W. Hardisty and I. C. Potter (eds.). Academic Press, London.

Stauffer, T. M. 1962. Duration of larval life of sea lampreys in Carp Lake River, Michigan. Trans. Am. Fish. Soc. 91: 422--423.

Vladykov, V. D. 1951. Fecundity of Quebec Lampreys. Can. Fish. Cult. 10:1--14.

Wilkinson, L. 1990. SYSTAT: the system for statistics. SYSTAT, Evanston, Illinois.

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TABLE 1. - Proportion of female, male and atypical gonads observed in sea lamprey larvae (*Petromyzon marinus*), and the number of typical and atypical females sub-sampled from four study streams.

Stream	Sample		Percent Composition			Potential fecundity sample size	
	Date	Size	Female	Male	Atypical	Typical	Atypical
Cannon	1995	172	60	31	9	3	2
Gordon's	1995	290	3	15	82	0	3
	1996	80	36	50	14	1	5
Lynde	1995	119	67	14	19	4	2
Cobourg	1996	109	24	33	43	0	3

TABLE 2. - Morphology and condition of typical and four groups of atypical gonads from landlocked sea lamprey larvae (*Petromyzon marinus*) collected from streams tributary to the Great Lakes.

Gonad type	Total length (mm)	Sample size	Gonad morphology and composition
Typical female	115-165	8	horseshoe-shaped, prominent lobes, no atresia, second stage oocytes only, oocytes arranged in pairs
Atypical 1	120-129	4	angular-shaped, no lobes, no atresia, 1st stage oocytes only, oocytes only present around perimeter of gonad
Atypical 2	116-137	4	horseshoe-shaped, no lobes, atresia (2,000-10,000), 2nd stage oocytes only, many germ cell clusters, oocytes scattered individually
Atypical 3	122-146	3	horseshoe-shaped, lobes, no atresia, excess stromal tissue and few oocytes in some lobes
Atypical 4	119-141	4	horseshoe-shaped, lobes, atresia (5,000-40,000), many germ cell clusters, 1st and 2nd stage oocytes

TABLE 3. - Quantitative characteristics of typical gonads and the four groups of atypical gonads measured on sea lamprey larvae (*Petromyzon marinus*) collected from streams tributary to the Great Lakes. Values given are ranges over the total lengths examined.

Characteristic	Gonad Type				
	Typical	Atypical			
		1	2	3	4
Germ cells	0.5--80	0--27	10--5300	0--20	27--4100
Number ($\cdot 10^3$)					
Oocytes					
Number ($\cdot 10^3$)	19--65	12--40	8--51	22--39	19--25
Diameter (μm)	56--88	15--18	33--46	52--58	39--56
Gonad					
Length (mm)	50--64	49--57	48--57	44--56	48--58
Area (mm^2)	32--117	4--6	14--63	39--72	20--56
($\cdot 10^{-2}$)					

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Figure 1. Transverse sections from the mid-region of larval sea lamprey, *Petromyzon marinus*. (A) A horseshoe shaped gonad from a 123 mm larva from Cannon Creek collected in 1995; oocytes are uniform in size, and there is no evidence of atresia (100X). (B) An atypical gonad from a 120 mm larva from Gordon's Creek in 1995; the gonad shows first stage oocytes, arranged around the perimeter of the angular shaped gonad (100X). (C) An atypical gonad from a 116 mm larva from Cobourg Brook (1996); the gonad shows the large germ cell clusters (g) and very few oocytes (100X). (D) An atypical female gonad from a 124 mm larva from Gordon's Creek (1996); the gonad shows the large amount of stromal tissue (s) (200X). (E) An atypical gonad from a 141 mm larva from Gordon's Creek (1996); the gonad shows a high proportion of vacuolated oocytes, or atresia (a), as well as normal oocytes (o) and several germ cell clusters (g) (400X). (F) An atypical gonad from a 119 mm larva from Lynde Creek (1995); the gonad shows faded, spongy oocytes, or atresia (a), in addition to typical oocytes (o) (400X).

FIGURE 1 LAB, BJM, BJW, FWHB

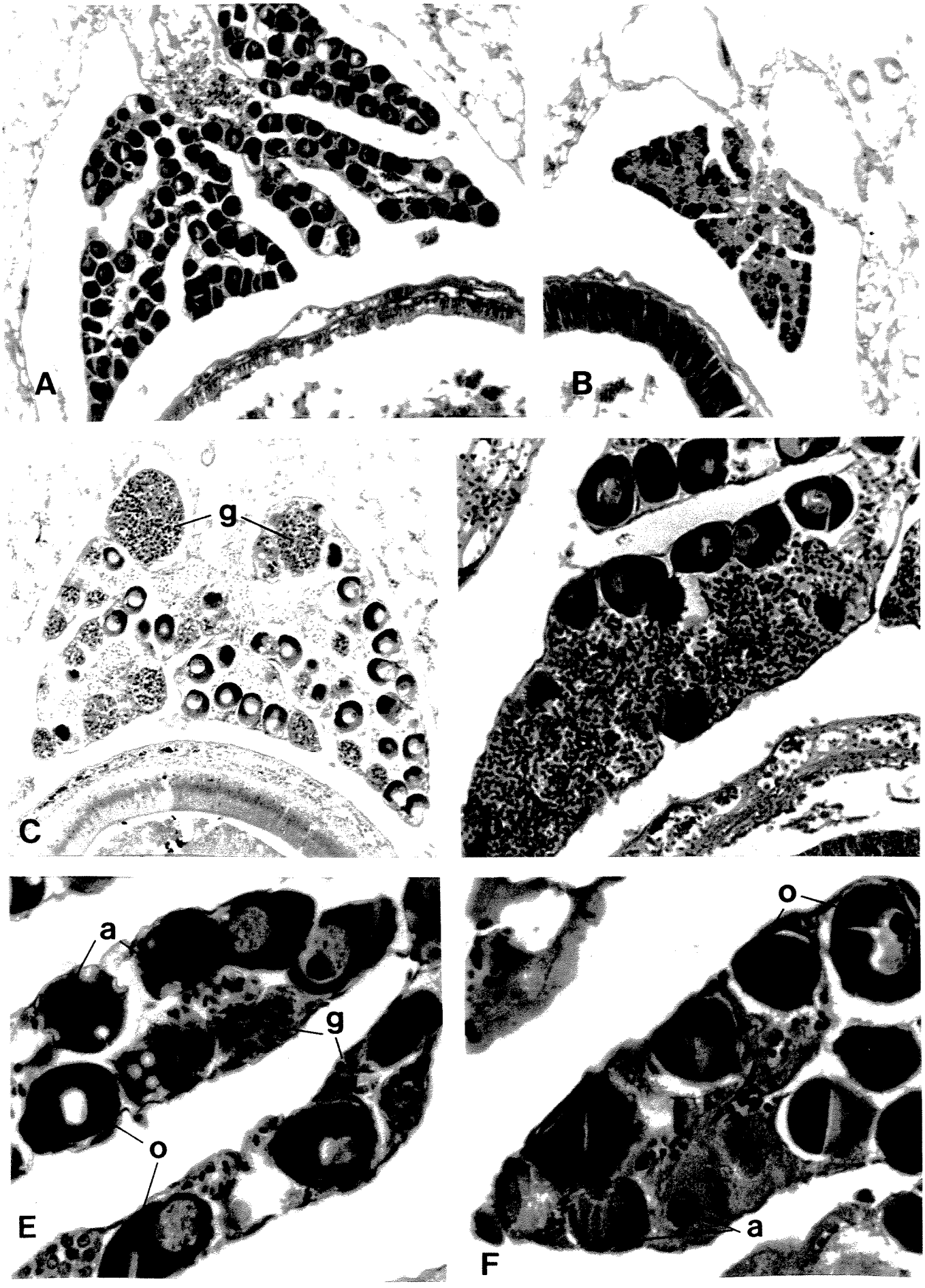

Figure 1