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An atlas of reproductive development in rockfishes, genus *Sebastes*

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Abbreviations

Abbreviations associated with spermatogenesis (Figs. 1–11):

ESd	early spermatid	Ld	Leidig cell
ESz	early spermatozoa	LL	lobule lumen
Flag	flagella	LSd	late spermatid
Ga	primary (1°) spermatogonia	LSz	late spermatozoa
Gb	secondary (2°) spermatogonia	PSc	primary (1°) spermatocyte
Gc	tertiary (3°) spermatogonia	RSz	residual spermatozoa
Gd	quaternary (4°) spermatogonia	Sr	Sertoli cell
IT	interstitial tissue	SSc	secondary (2°) spermatocyte

Abbreviations associated with ovarian development (Figs. 12–24):

CN	chromatin nucleolus	Og	oogonium
EF	empty follicle=post-ovulatory follicle	ON	oogonial nest
EL	eyed larvae	OV	oil vacuole
Em	embryo	PM	prematuration oocyte
EPN	early perinucleus	PY	primary (1°) yolk
FC	follicle cell	REm	residual embryo
GR	granulosa	So	somite
IT	interstitial tissue	SY	secondary (2°) yolk
LC	lampbrush chromosome	Sz	stored spermatozoa
LPN	late perinucleus	TY	tertiary (3°) yolk
MN	migratory nucleus	Y	yolk
MO	mature oocyte	YD	yolk droplets
N	nucleus	ZR	zona radiata
No	nucleolus		

Abbreviations associated with embryogenesis (Figs. 25–59):

AP	auditory placode	M	mouth opening
Bd	blastodisc	NC	nerve chord
Bm	blastomere	Noto	notochord
Br	brain	OV	oil vacuole
CM	chorion membrane	Ot	otolith
DT	digestive tract	OlfV	olfactory vesicle
ES	embryonic shield	OpC	optic cup
EVL	outer enveloping layer	OpV	optic vesicle
Ey	eye	PA	pharyngeal arches
FF	fin fold	PF	pectoral fin
GR	granulosa	PG	pigment granules
He	heart	PL	pigment layer
HF	head fold	So	somite
L	lens	Y	yolk
Mec	Meckel's cartilages	YSL	yolk syncytial layer
MF	mitotic figures		

Abstract—The genus *Sebastes* consists of over 100 fish species, all of which are viviparous and long-lived. Previous studies have presented schemes on the reproductive biology of a single targeted species of the genus *Sebastes*, but all appear to possess a similar reproductive biology as evidenced by this and other studies. This atlas stages major events during spermatogenesis, oogenesis, and embryogenesis, including atresia, in six species of *Sebastes* (*S. alutus, S. elongatus, S. helvomaculatus, S. polyspinis, S. proriger*, and *S. zacentrus*). Our study suggests that the male reproductive cycle of *Sebastes* is characterized by 11 phases of testicular development, with 10 stages of sperm development and 1 stage of spermatozoa atresia. Ovarian development was divided into 12 phases, with 10 stages of oocyte development, 1 stage of embryonic development, and 1 stage of oocyte atresia. Embryonic development up to parturition was divided into 33 stages following the research of Yamada and Kusakari (1991). Reproductive development of all six species examined followed the developmental classifications listed above which may apply to all species of *Sebastes* regardless of the number of broods produced annually. Multiple brooders vary in that not all ova are fertilized and progress to embryos; a proportion of ova are arrested at the pre-vitellogenic stage. Reproductive stage examples shown in this atlas use *S. elongatus* for spermatic development, *S. proriger* for oocyte development, and *S. alutus* for embryological development, because opportunistic sampling only permitted complete analysis of each respective developmental phase for those species. The results of this study and the proposed reproductive phases complement the recommended scheme submitted by Brown-Peterson et al. (2011), who call for a standardization of terminology for describing reproductive development of fishes.

An atlas of reproductive development in rockfishes, genus *Sebastes*

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Introduction

The genus *Sebastes* is a prolific, species-rich, and important commercial fish taxon in the Northeast Pacific Ocean. Over 100 species of the genus *Sebastes* exist worldwide, occupying a wide range of habitats from intertidal to depths >1000 m (Haldorson and Love, 1991; Kendall, 1991). Seventy-two species occur in the Northeast Pacific; however, only a few Pacific species of the genus *Sebastes* (e.g., *S. alutus*, *S. caurinus*, *S. flavidus*, and *S. schlegeli*) have been well studied. There is limited information on *S. elongatus* (greenstriped rockfish), *S. helvomaculatus* (rosethorn rockfish), *S. proriger* (redstripe rockfish), and *S. zacentrus* (sharpchin rockfish), particularly concerning their distribution, abundance, and life history parameters. These four species, plus *S. alutus* and *S. polyspinis*, are found in an overlapping geographic region (i.e., the Northeast Pacific), but vary with respect to distribution range (Orr et al., 2000; Love et al., 2002) and depth. In a study of *Sebastes* spp. assemblages (i.e., rockfish species captured during West Coast assessment surveys), Weinberg (1994) determined that several of these six species co-occur in distribution a significant amount of the time.

The above rockfish species, with the exception of *S. alutus* and *S. polyspinis*, have been lightly exploited in the past, but can be captured efficiently with bottom trawls. With declines in populations of other commercial fish species, their importance is growing as the fishing industry seeks new fishing opportunities. Landings of these four rockfish species have already begun to increase (Douglas, 1998; Dick and MacCall, 2010), and information on life history parameters is critical for their management. Currently these four rockfish species are managed with other *Sebastes* species under a "*Sebastes* complex" quota and listed variously as "small rockfish," "other rockfish," or "remaining" rockfish in the catch statistics.

The genus *Sebastes* is in the subfamily Sebastinae, and family Scorpaenidae (Kendall, 1991). *Sebastes* and the other three genera in the subfamily Sebastinae are all live-bearing (or viviparous), while most other genera in the family Scorpaenidae are oviparous (Takahashi et al., 1991). Fertilization occurs internally in *Sebastes*; embryos develop, hatch in the ovaries, and are extruded as larvae.

Our study suggests that oocyte development for each respective species is group synchronous, producing only one brood per year (Asturiano et al., 2002), although there is evidence to indicate that under certain environmental conditions and food

availability, some species (i.e., *S. elongatus*) are capable of producing multiple broods (Love et al., 1990). After ovarian recrudescence is well underway, two modes of oocytes are found: one mode at resting stage and one progressing synchronously through vitellogenesis or maturation. In *Sebastes* there is a delay between copulation and oocyte fertilization during which sperm is stored in the inter-lamellar tissues of a mature ovary (Moser, 1967a). As a result, the reproductive development of males and females is asynchronous temporally. The mating season precedes the maturation of the oocytes, imposing a need for sperm storage. Copulation can precede oocyte maturation by as long as six months (Moser, 1967a, 1967b; Shaw, 1999).

Research detailing sexual maturity in *Sebastes* has included a combination of several methods. A number of studies were limited to gross anatomical criteria for staging of the ovaries. Sometimes a gonadosomatic index was used as a measure of sexual maturity. Microscopic analysis and complete oocyte staging of rockfishes is often incomplete. Even less has been done to document sperm development in *Sebastes*. The presented observations will contribute to a better understanding of reproduction in this genus by providing additional and more detailed descriptions of this process.

We present in this paper a histological scheme to divide the reproductive cycle of *Sebastes* into 11 phases of male reproductive development, with 10 stages of sperm development and 1 stage of spermatozoa atresia. Female reproductive development was divided into 12 phases, with 10 stages of oocyte development, 1 stage of embryonic development, and 1 stage of oocyte atresia. Embryonic development up to parturition was divided into 33 stages. The developmental phases of all species reviewed in this histological study (*S. alutus, S. elongatus, S. helvomaculatus, S. polyspinis, S. proriger*, and *S. zacentrus*) followed the developmental classifications listed above. As a result, the proposed classifications may apply to all *Sebastes* species regardless of the number of broods produced per year; some species from Southern California and farther south have more than one brood per year (Moser, 1967b; Love et al., 1990). In this study, because of completeness of sample collection for some species, *S. elongatus* is the standard example for sperm development at the microscopic level, while *S. proriger* and *S. alutus* serve as similar standards for oocyte and embryological development, respectively.

This study blended together existing information on rockfish life history parameters, examined existing data sets, and made special collections to fill data gaps to assemble a complete description of the life histories of the six targeted rockfish species. The mortality rates, age and size composition, depth and geographic distribution, food habits, and habitat requirements for four of these species were examined in order to determine if they can be managed as a group (Shaw, 1999; Shaw and Gunderson, 2006, 2008). All of this information is necessary to determine the productivity and resilience of these stocks. This information, along with responsible management, will enable long term exploitation of these species without endangering their populations or diminishing their role within their respective ecosystems. This atlas focuses only upon the reproductive biology of the above six rockfish species, while specific information addressing 50% maturity, age-length relationships, weight-length relationships, natural mortality, and total mortality for *S. elongatus*, *S. helvomaculatus*, *S. proriger*, and *S. zacentrus* can be found elsewhere (Shaw, 1999; Shaw and Gunderson, 2006, 2008).

Materials and methods

Collections at the rate of 15 specimens per species per month were targeted from January to October 1995 from commercial catches for *S. elongatus* (n=123), *S. helvomaculatus* (n=82), *S. proriger* (n=115), and *S. zacentrus* (n=124) landed at Astoria, OR. Under the collection scenario for these four species, fish were captured in bottom trawls at locations within a few hours travel from Astoria (near the mouth of the Columbia River). Specimens of *S. alutus* (n=172) and *S. polyspinis* (n=323) were collected primarily by fisheries observers aboard commercial trawlers, but also by Alaska Fisheries Science Center personnel on research cruises in Alaska waters from October 2003 to March 2004. Physical data collected at the time of capture included fork length to the nearest millimeter, somatic weight to the nearest gram, and gonad weight to the nearest tenth of gram; maturity stage of both male and female fish was visually determined. A total of 939 gonads were collected.

Both gonads (ovaries and testes) were excised whole from fish. Depending upon the mass of the gonad, several small incisions were made throughout the gonad. The incisions were intended to allow more rapid and thorough penetration of fixative into the gonad, without severely damaging the integrity of the gonad or excessive loss of representative gonadal cells. A label was prepared containing species, sex, size, haul number, and location (unless specimen and catch position data was proprietary information, as is the nature of fisheries observer data). The label and entire gonad were then placed in linen bags and preserved in 10% formalin buffered with sodium acetate trihydrate.

At the laboratory, small longitudinal cross-sections encompassing the anterior, middle, and posterior portion of the ovaries and testes were taken to ensure organ homogeneity. A small segment ($0.5 \times 0.5 \times 1.0$ cm^3) of tissue was excised from the center portion of each section of organ sampled. Tissue sections were prepared

for histological examination as noted by Sheehan and Hrapchak (1980). In brief, tissues were thin-sectioned with a rotary microtome to a thickness of 5 µm and placed on glass slides. Sections were de-paraffinized, passed through a graded ethanol series, stained with Harris' haematoxylin and eosin (H&E), and cover slipped. All slides were read blind, that is without prior knowledge of species, sex, size, or maturity.

Hematoxylin and eosin are stains routinely used within a histology laboratory. Hematoxylin possesses a deep blue-purple color and stains nucleic acids (i.e., DNA, RNA) and other acidic compounds by a complex reaction that is not entirely understood. Eosin is pink, and stains proteins and other basic compounds non-specifically and to varying degrees of intensity. In typical H&E stained tissue, nuclei are stained blue and the cytoplasm and extracellular matrix have varying degrees of pink staining. Not surprisingly, depending upon the state of the cell, the bluish nature or content of cells can vary considerably as RNA may be abundant within the cytoplasm of immature cells.

The size of the most advanced oocyte was used as a measure of ovary development (Clark, 1934). The diameter of the fifth largest oocyte in a random cross section from the middle portion of each ovary was used to represent the most advanced mode. Several investigators have used the diameter of the largest single oocyte to represent the most advanced mode (Yuen, 1955; Bunag, 1956; Otsu and Uchida, 1959). West (1990) reported that in unpublished studies at the Commonwealth Scientific and Industrial Research Organisation (CSIRO) Marine Laboratories on the threadfin bream (*Nemipterus furcosus*), correlation to the most advanced mode was improved from 0.97 to 0.99 ($P<0.01$) when the fifth largest oocyte was used, rather than the largest. This eliminated the situation where the largest oocyte might be an outlier from the distribution. In his comprehensive review of methods of assessing ovarian development, West (1990) found "little point in using measures more complicated than maximum (largest) oocyte diameter."

Oocyte and embryo diameters were measured from histological cross sections, using an ocular micrometer and compound microscope. If embryos were present in the ovary or if embryos had hatched, an estimated standard length of the larvae was calculated using image analysis software.

Morphological observations

Collections of *S. elongatus, S. helvomaculatus, S. proriger*, and *S. zacentrus* were well distributed across all sizes, representing late immature to mature phases for both males and females. Collections of *Sebastes alutus* and *S. polyspinis* were not sufficiently distributed to provide a good overview of gonad development for these two species. The large majority of collections were of mature females harboring embryos and mature males post-spawning. As a result, *S. alutus* was used as the "model" for embryonic development. Small numbers of representatives of other developmental phases were collected that permitted general agreement with similar gonad developmental phases with the other four species.

Gonadal development of these six species was similar to that reported for most other northern species of *Sebastes* (Moser, 1967a, 1967b; Leaman, 1988; Nichol, 1990; Takahashi et al., 1991; McDermott, 1994). Under the sampling scheme in this study, reproductive development is generally group-synchronous, producing only one brood per year. However, under different environmental conditions, *S. elongatus* is capable of producing multiple broods (Love et al., 1990). After ovary recrudescence was well underway, two modes of oocyte stages were found, one mode at resting or pre-vitellogenic stages and one synchronized at a vitellogenic or maturation stage. The indices of gonadal development (GSI, ovary phase, and oocyte diameter in females; GSI and testis phase in males) produced similar results except that the peak in the GSI was slightly earlier than the other indices (see Table 1). This was due to the release of spermatozoa or embryos as the spawning season progressed.

The reproductive development of males and females is offset temporally (e.g., male gonad development anticipates female development). As a result, the mating season precedes the maturation of the oocytes, imposing a need for sperm storage (Fig. 17), while increasing the probability that spermatozoa will be available when oocyte maturation has occurred. Copulation preceded oocyte maturation by a minimum of one month in *S. helvomaculatus* to as long as six months in *S. proriger*. Parturition occurs in the late spring for four of the six examined species (Table 1).

We prefer to use the term "phase," as opposed to "class," to describe visual reproductive organ development and its aligned microscopic features because the term implies transition and not rigid categories. Indeed, a phase may be represented by several cell stages. The term "stage" is used to identify cellular development within both male and female reproductive organs, while the term "interval" refers to a group of related stages within an organ phase. Using this terminology, gross developmental phases of the ovary (5) and testes (4) were fewer in number than histological phases and therefore less precise in reflecting gonad status. Visual accuracy was also a problem. Immature phases were often difficult to differentiate from late spent or resting phases in both sexes, and misclassifications sometimes occurred. To assess maturity without histological confirmation, gross observations should be made during the mating season (late fall to winter) for

Table 1

Summary of life history parameters for *Sebastes elongatus*, *S. helvomaculatus*, *S. proriger,* and *S. zacentrus*. Similar data not available for *S. polyspinis*. Similar data for *S. alutus* can be found in Haldorson and Love (1991). Data for *S. elongatus* and *S. helvomaculatus* presented with permission from Shaw and Gunderson (2006, 2008).

	elongatus		*helvomaculatus*		*proriger*		*zacentrus*	
Parameter	♂	♀	♂	♀	♂	♀	♂	♀
Max. age[1]	45	46	64	61	34	28	30	41
Max. length[2]	430	400	400	376	440	520	450	420
Max. weight[3]	980	915	910	760	1060	1700	1130	1035
L_∞ (Wetherall)[4]	324	378	292	300	334	377	291	367
L_∞ (Bertalanffy)[5]	301	375	279	287	295	383	269	332
K[6]	0.11	0.08	0.11	0.10	0.22	0.16	0.20	0.17
t_0[7]	–3.27	–3.45	–2.07	–2.78	-0.72	–0.79	–0.81	–0.68
$L_{0.5}$[8]	230	210	228	208	243	262	209	223
$A_{0.5}$[9]	10	7	13	10	7	7	7	6
M_1[10]	0.10	0.10	0.07	0.07	0.13	0.16	0.15	0.11
M_2[11]	0.10	0.12	0.04	0.05	0.10	0.17	0.14	0.12
M_3[12]		0.15		0.04		0.13		0.12
Z[13]	0.43	0.31	0.35	0.39	0.95	0.62	0.70	0.73
GSI[14]	0.8	8.3	0.6	2.3	1.1	7.3	0.9	6.6
Mating[15]	Dec–Feb		Dec–Apr		Nov–Jan		Oct–Jan	
Parturition[16]	June		May–Jun		June		Apr–May	
Gestation[17]	40–50		40–60		35–50		40–60	
Max. depth[18]	491		549		425		444	
Mean depth[19]	158		219		156		207	
Range north[20]	59°52′N		59°38′N		59°59′N		59°59′N	
Range south[21]	28°10′N		32°34′N		32°43′N		32°46′N	

[1]Maximum age in years.
[2]Maximum fork length in mm.
[3]Maximum weight in grams.
[4]Theoretical maximum length in mm calculated from Wetherall plot.
[5]Theoretical maximum length in mm calculated from von Bertalanffy growth model.
[6]Von Bertalanffy growth coefficient.
[7]Theoretical age in years at zero length calculated from von Bertalanffy growth coefficient.
[8]Von Bertalanffy estimated length in mm at 50% maturity.
[9]Von Bertalanffy estimated age in years at 50% maturity.
[10]Hoenig maximum age based estimated mortality rate.
[11]estimated mortality rate from catch data.
[12]estimated mortality rate from calculated gonadosomatic index (GSI).
[13]Wetherall estimated total mortality.
[14]Gonadosomatic index.
[15]Period of mating.
[16]Spawning period.
[17]Gestation period in days.
[18]Maximum depth distribution in m.
[19]Mean depth of distribution.
[20]Northern range of distribution.
[21]Southern range of distribution.

males and at the time of parturition (late spring) for females since no misclassifications were made of fish sampled at these times.

Finally, we wish to emphasize that inconsistent terminology presents complications in describing fish reproductive development as evidenced in a recent paper by Brown-Peterson et al. (2011). We have adopted the general terminology and organ development convention of Brown-Peterson et al. (2011) and Parenti and Grier (2004) in this atlas although we recognize the current momentum to use higher vertebrate terminology in describing fish germ cells in spermatogenesis and

oogenesis (Lubzens et al., 2010; Schultz et al., 2010). Citing previous research, these studies have done much to standardize terminology by careful description of cell morphology and function. For example, the term "lobule boundary cell" is no longer a valid term as considerable confusion existed in the location and function of this cell-type (Grier, 1993; Schulz et al., 2010). Furthermore, the testis appears to be of the unrestricted lobular type as defined by Grier (1993).

Testis development

The *Sebastes* testes are paired, elongate structures suspended dorsally in the posterior portion of the peritoneal cavity by a mesorchium. The two lobes of the testis are solid in appearance and equal in size, and cross-sections are roughly triangular except at the anterior and posterior ends. The efferent ducts of each lobe empty posteriorly and fuse to form a common seminal duct, which continues posteriorly between the urinary tract and intestine. It communicates exteriorly through a pore in the tip of the urogenital papilla.

Spermatogenesis is the transformation of primary spermatogonia into mobile spermatozoa through a series of cell divisions, both meiotic and mitotic. In the adult testis spermatogonial cysts develop along the entire length of the lobule. The cells within a cyst are derived from a single spermatogonium and thus represent a clone that develops synchronously (Schultz et al., 2010). However, within a lobule, spermatogonial cysts may develop asynchronously. Spermatocytes are formed as the spermatogonia enter meiotic prophase; spermatids are the result of the second meiotic division, and spermatozoa result from reorganization of the nucleus and cytoplasm and the formation of the flagellum. Spermatogenesis is dvided into 10 stages of cellular development following Carrillo and Zanuy (1977) and Gordo (1995) for stages of spermatogonia, and Agarwal (1996) with slight modification (spermatid and spermatozoa stages were split into early and late stages for each) for the remaining spermatogenesis stages.

Spermatogonia

Stage i: 1° spermatogonia (8–11 µm)

Primary spermatogonia (Ga) are the largest and most segregated among the spermatogenic cells. They possess a large, round, light-staining nucleus with a prominent nucleolus and chromatin threads. The cytoplasm has a weak affinity for basic dyes. They are found in higher concentration on the periphery of the testis and near the lobular wall, which is lined by Sertoli cells. All stages of spermatogonia have a diploid set of chromosomes.

Stage ii: 2° spermatogonia (6–8 µm) (Fig. 1)

Secondary spermatogonia (Gb) are derived from dividing primary spermatogonia and are found in small groups. The nucleus shows moderate staining with visible chromatin threads. They possess a large nucleus to cytoplasm ratio.

Stage iii: 3° spermatogonia (4–6 µm) (Fig. 2)

Tertiary spermatogonia (Gc) possess a condensed nucleus that stains dark due to the increasing density of the chromatin. They possess a reduced nuclear to cytoplasm ratio and are found in larger numbers than secondary spermatogonia within the Sertoli cell lined cysts.

Stage iv: 4° spermatogonia (2.5–4 µm) (Fig. 3)

Quaternary spermatogonia (Gd) have a more condensed nucleus with less cytoplasm and are found in even larger groups than the previous stage.

Spermatocytes

Stage v: 1° spermatocytes (3–4.5 µm) (Fig. 4)

A short growth phase follows a quiescent period that gives rise directly to primary spermatocytes (PSc). A series of nuclear transformations (meiotic prophase) signals the first meiotic division. Cells possess a large basophilic nucleus and a large nucleus to cytoplasm ratio.

Stage vi: 2° spermatocytes (2–2.5 µm) (Fig. 5)

Each primary spermatocyte undergoes the first meiotic, or reduction, division and produces two daughter cells called secondary spermatocytes (SSc). These cells are very round and have a dark-staining nucleus but have a smaller nucleus to cytoplasm ratio than the previous stage.

Spermatids

Stage vii: early spermatids (2 µm) (Fig. 6)

The secondary spermatocytes undergo a second meiotic division, which gives rise to early spermatids (ESd) that now have a haploid set of chromosomes. In early spermatids the cytoplasm is difficult to see and the dark-staining nucleus is found in the periphery of the cell. The nucleus has a half moon shape early in this stage and later becomes crescent-shaped, which covers more than 180° of the circumference of the round cell.

Stage viii: late spermatids (2–2.5 µm) (Fig. 7)

In late spermatids (LSd) the crescent-shaped nucleus straightens to become more fusiform. This causes the shape of the cell to change from round to oval. The cell wall subsequently disappears.

Spermatozoa

Stage ix: early spermatozoa (10–12 µm including the flagellum) (Fig. 8)

Spermatids undergo a process of differentiation, giving rise to the mobile spermatozoa. Early spermatozoa (ESz) possess a long flagellum with a long pyriform-shaped nucleus (~3 µm in length and 1 µm in diameter) that are deeply staining but remain within cysts of the seminiferous tubule, with their heads embedded in the Sertoli cells.

Stage x: late spermatozoa (10–12 µm including the flagellum) (Fig. 9)

Late spermatozoa (LSz) are free of the cyst and begin to migrate into the tubule lumen of the spermatic duct, free from the Sertoli cells.

Atresia

Stage α: residual spermatozoa (Figs. 10 and 11)

Some spermatozoa remain in some lobules after spawning. Sertoli cells are often observed attached to or with engulfed residual spermatozoa (RSz), demonstrating their important role in resorption or phagocytosis of residual spermatozoa.

Testis phases

Ten testis development phases and one atresia phase (i.e., organ development that may contain several sperm cell types or stages) closely parallel spermatocyte stages (i.e., specific cellular development of testicular cells). Each phase is defined as beginning with the first appearance of the same stage spermatocyte and ending with the first appearance of the next stage spermatocyte in the testis as a whole. The ten development phases are followed by one atresia (or spent) phase. The various phases will be illustrated in the figures with sections from *S. elongatus* testes.

Phase I: 1° spermatogonia

No testes were found in this phase even though fish as small as 210 mm and testis that weighed less than 1 g were examined.

Phase II: 2° spermatogonia (Fig. 1)

Testes in this phase retained primary and secondary spermatogonia. Figure 1 illustrates a testis in this phase from a fish that has never spawned. Testes of this phase are considered immature unless there is evidence of residual spermatozoa (RSz). Sperm cells in mature testes progress past the secondary spermatogonia stage before all the RSz are resorbed.

Phase III: 3° spermatogonia (Fig. 2)

Phase III testes possess primary, secondary, and tertiary spermatogonia. Primary spermatogonia stain the weakest and are found singularly. Secondary spermatogonia are found in groups of 3–5, while tertiary spermatogonia are found in groups of 10–20 per cyst and stain the darkest with H&E stain.

Phase IV: 4° spermatogonia (Fig. 3)

All four stages of spermatogonia are contained in testes of this phase. Quaternary spermatogonia stain the darkest of the spermatogonia. The population of quaternary spermatogonia in each cyst is about twice that of tertiary spermatogonia, although actual counts are difficult. This was due to the combination of the shrinking size of the spermatogonia and the 3-dimensional nature of the cysts, where the 2-dimensional section does not reveal all of the spermatogonia within each cyst.

Phase V: 1° spermatocytes (Fig. 4)

Testes in this phase contain all four stages of spermatogonia plus the primary spermatocyte. Stained testes sections (H&E) have a noticeably darker appearance as the sperm cells begin to dominate.

Phase VI: 2° spermatocytes (Fig. 5)

Secondary spermatocytes are very round, dark, and about twice as numerous per cyst as the primary spermatocytes. This phase of testis development contains primary spermatogonia and sometimes other stages of spermatogonia.

Phase VII: early spermatids (Fig. 6)

This phase of testes stains very dark with H&E, with early spermatids and secondary spermatocytes dominating. Primary spermatogonia and other stages of spermatogonia are diminished in number and take on the role of the "reserve fund."

Phase VIII: late spermatids (Fig. 7)

Nearly all the cysts have developed past the secondary spermatocyte stage. The crescent-shaped early spermatids and the "pancaking" late spermatids dominate. Very few spermatogonia are noticeable.

Phase IX: early spermatozoa (Fig. 8)

The pink to light red flagella (Flag) (H&E stain) make their appearance at the center of the cyst with the heads of the early spermatozoa oriented to the perimeter.

Table 2

Summary of the gross and histological phases and cell stages of testes in *Sebastes*. Table partially reproduced and with permission from Shaw and Gunderson (2006, 2008).

Gross testes phase	Histological testes phase	Sperm cell stages present
Immature	I, II	Ga,Gb
Developing	III, IV, V, VI, VII, VIII, IX	Ga, Gb, Gc, Gd, PSc, SSc, ESd, LSd, ESz
Ripe	X	Ga, ESz, LSz
Spent	XI	RSz, LSz, PSc, SSc
Regenerating	XI, II, III, IV	RSz, Ga, Gb, Gc, Gd

Early and late spermatids and early spermatozoa are numerous while spermatogonia stages become rare.

Phase X: late spermatozoa (Fig. 9)

At this testis phase, all sperm cells within the cyst have arrived at the late spermatozoa stage. A few primary spermatogonia can be found among the cyst lining Sertoli cells. Late spermatozoa have moved into the efferent ductules and the spermatic duct. Here, they accumulate until mating occurs.

Phase XI: residual spermatozoa (Figs. 10 and 11)

Spermatogenesis occurs within the stoma (epithelium) of seminiferous tubules and as spermatogenesis progresses, that epithelium becomes attenuated (e.g., becomes shorter in height), yielding to the increasing number of developing spermatozoa. Eventually, the once robust cuboidal Sertoli cells become shorter in height as the mature and profuse spermatozoa fill the lumen. After mating and release of mature spermatozoa, the tubular epithelium of the seminiferous tubules begins a recovery process.

In this phase of the testes, the dark spermatozoa give way to the recovering Sertoli cells and interstitial tissue. A few pockets of residual spermatozoa can be found, often in the process of resorption which is generally facilitated by the Sertoli cells.

Gross morphological phases of the testes

Immature: Testes are small, translucent, and string-like, with sharp edges. No milt is present.

Developing: Testes are larger and opaque; milt is present.

Ripe: Testes are large and white in color. Milt is present in the rounded cross section and can be expressed with pressure to the abdominal cavity at the peak of this phase.

Spent: Testes are brown to gray in color, small and flaccid. No milt is present.

Regenerating: Testes become firm as the organ increases in mass, and become triangular in shape and translucent on the periphery.

In the field, visual examination of testis maturity provides an on-site, but potentially inaccurate, diagnosis of gonad development. Histological or microscopic analysis provides a more accurate and complete description of testis development, but is not as rapid. Comparison of the two methods is provided in Table 2, which summarizes tissue and cellular features associated with visually described testis development.

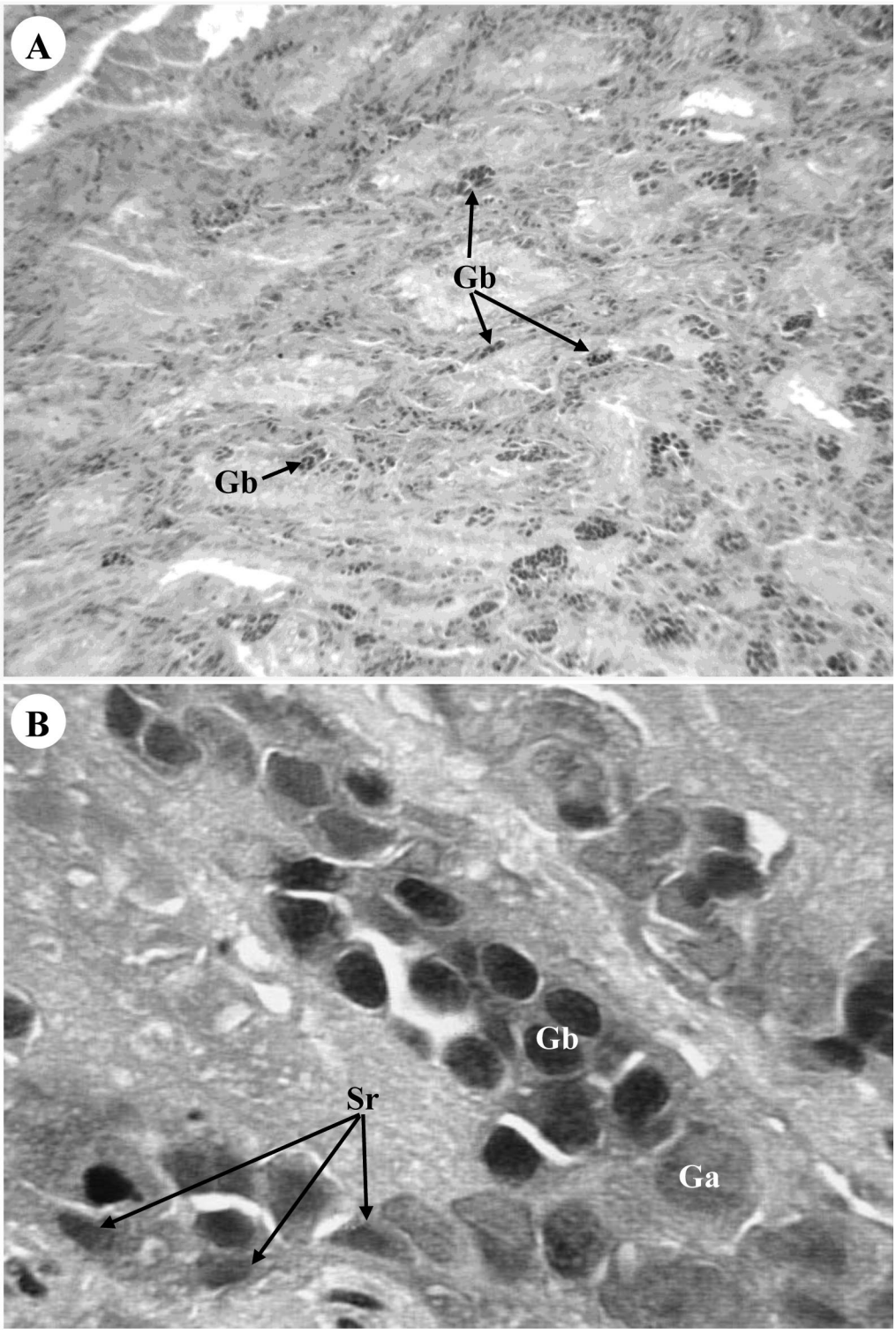

Figure 1

Phase II testes (secondary spermatogonia phase) of a 254 mm *Sebastes elongatus* with primary and secondary spermatogonia. Collected 10 June 1995. (A) 235× and (B) 2000×.

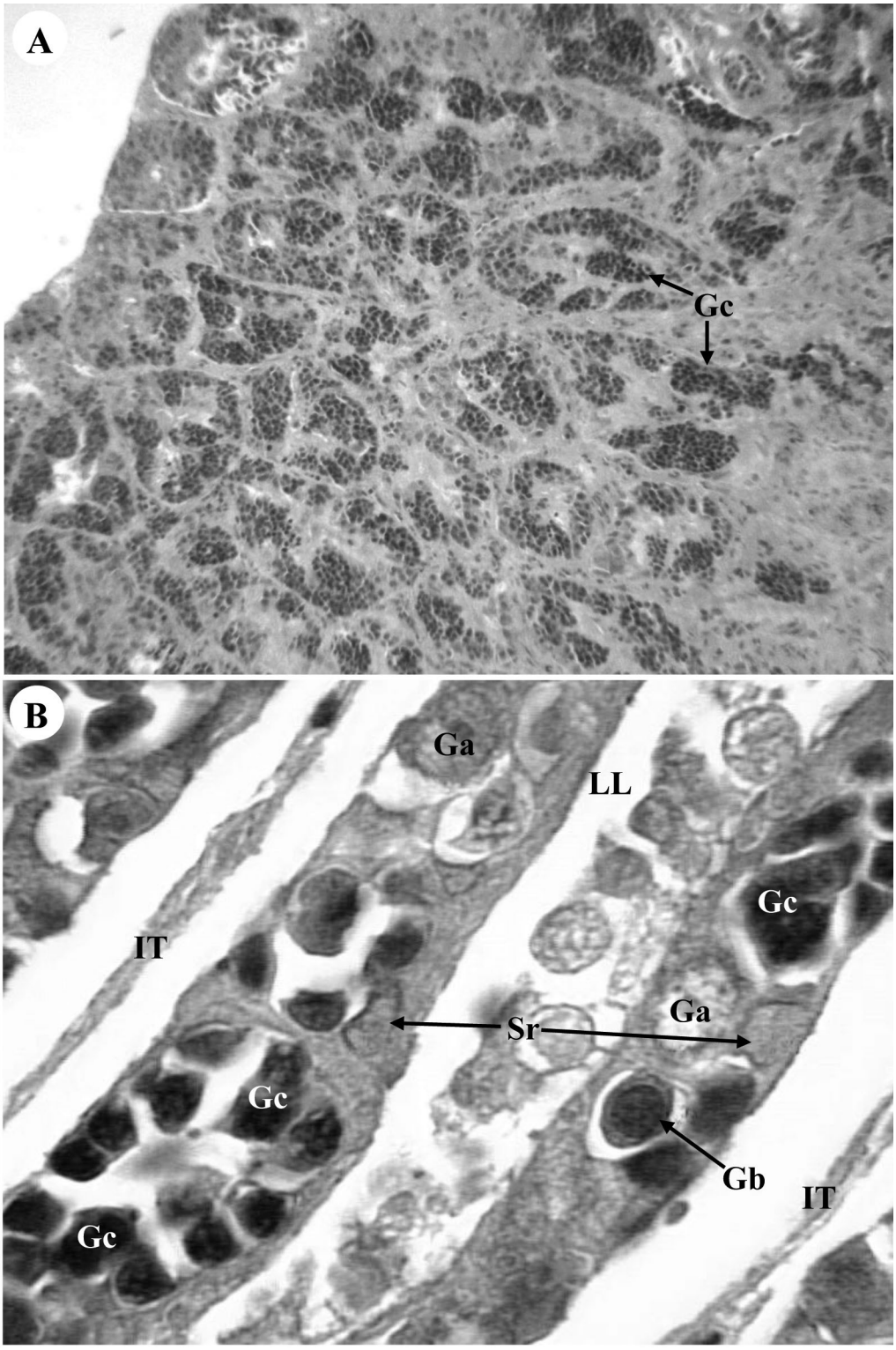

Figure 2

Phase III testes (tertiary spermatogonia phase) of a 266 mm *Sebastes elongatus* with primary, secondary, and tertiary spermatogonia. Collected 15 July 1995. (A) 235× and (B) 2000×.

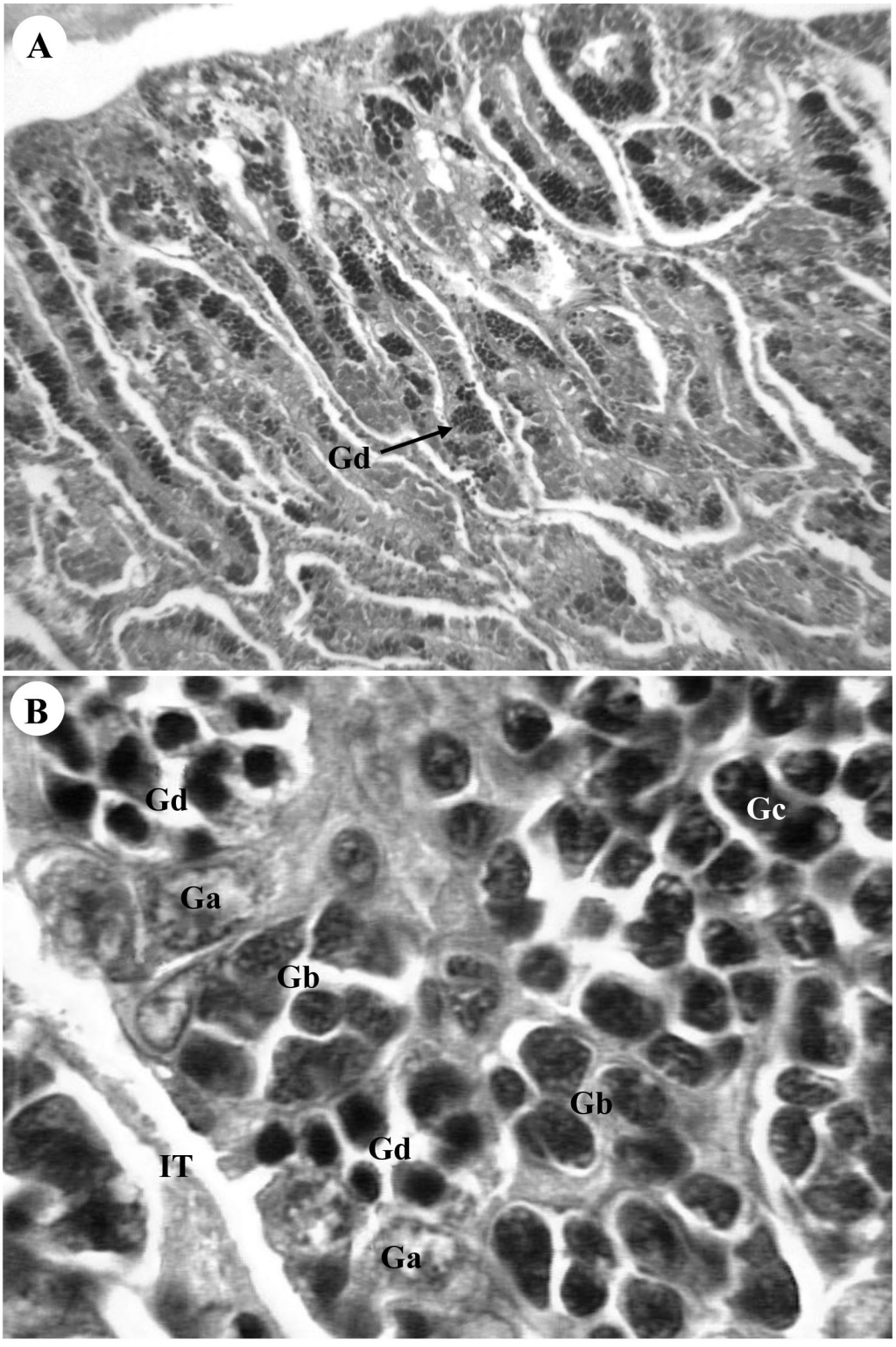

Figure 3

Phase IV testes (quaternary spermatogonia phase) of a 261 mm *Sebastes elongatus* with primary, secondary, tertiary, and quaternary spermatogonia. Collected 15 July 1995. (A) 235× and (B) 2000×.

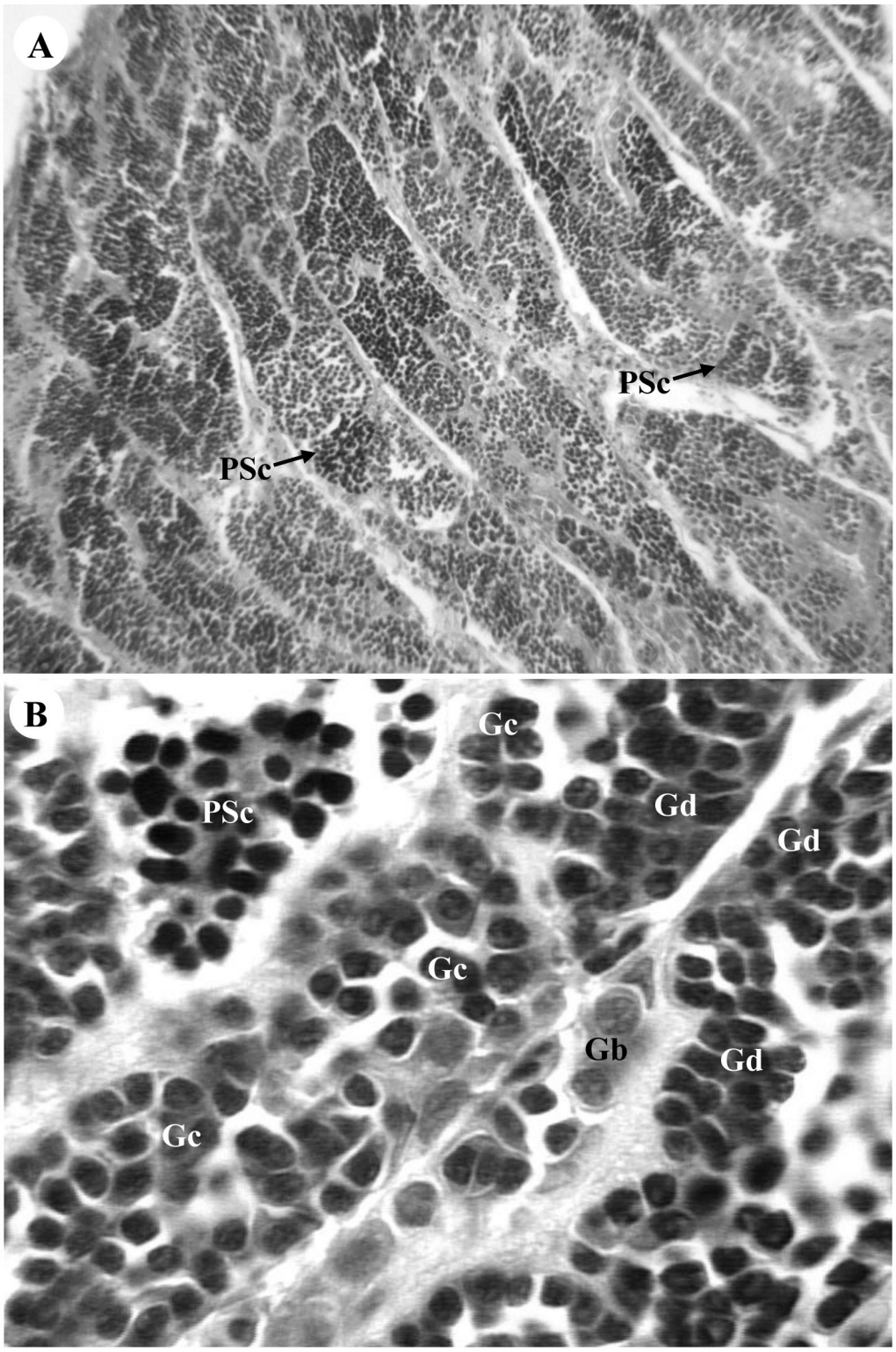

Figure 4

Phase V testes (primary spermatocyte phase) of a 279 mm *Sebastes elongatus* with primary, tertiary, and quaternary spermatogonia, and primary spermatocytes. Collected 18 August 1995. (A) 235× and (B) 2000×.

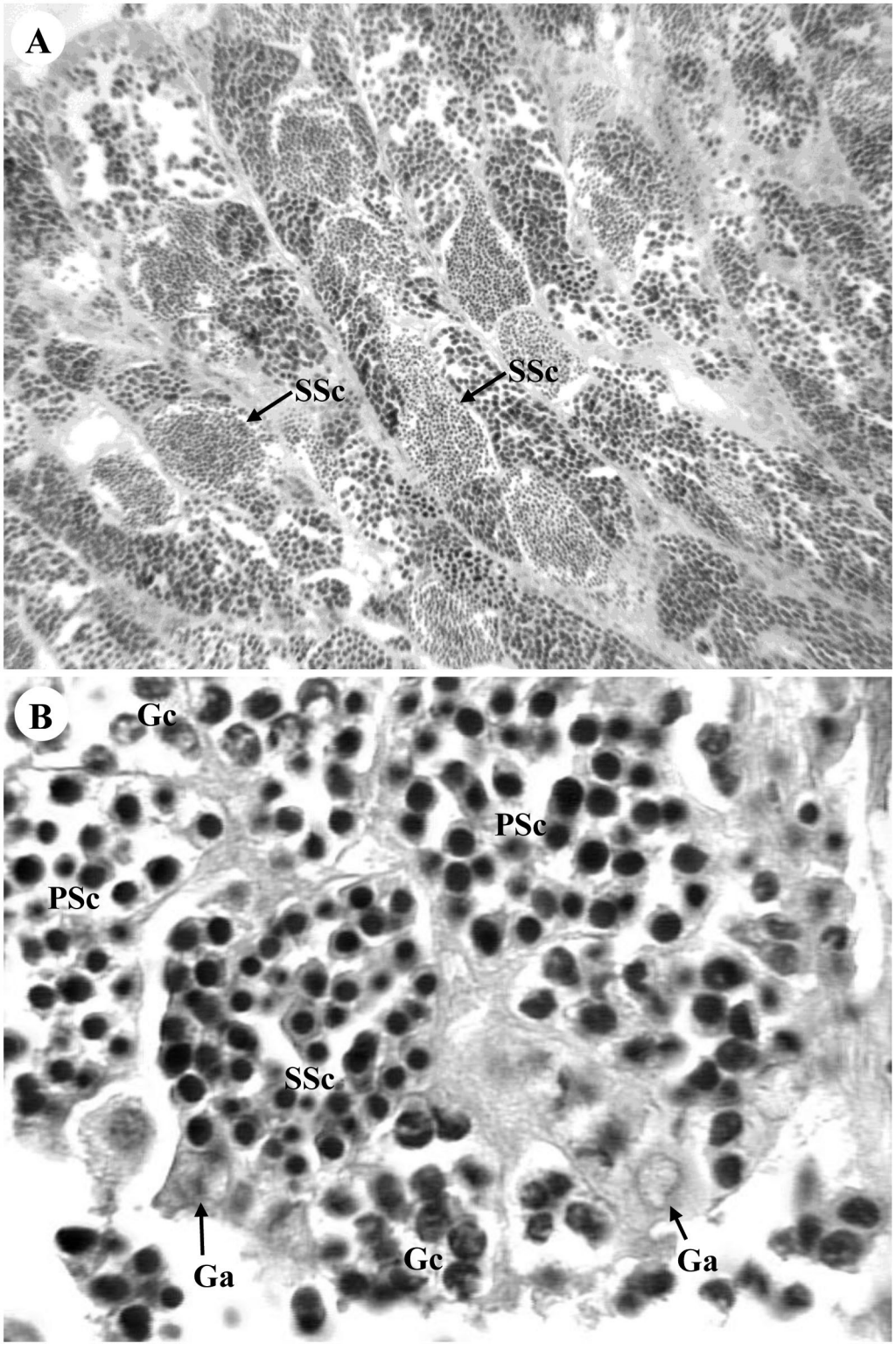

Figure 5

Phase VI testes (secondary spermatocyte phase) of a 293 mm *Sebastes elongatus* with primary and tertiary spermatogonia, and primary and secondary spermatocytes. Collected 18 August 1995. (A) 235× and (B) 2000×.

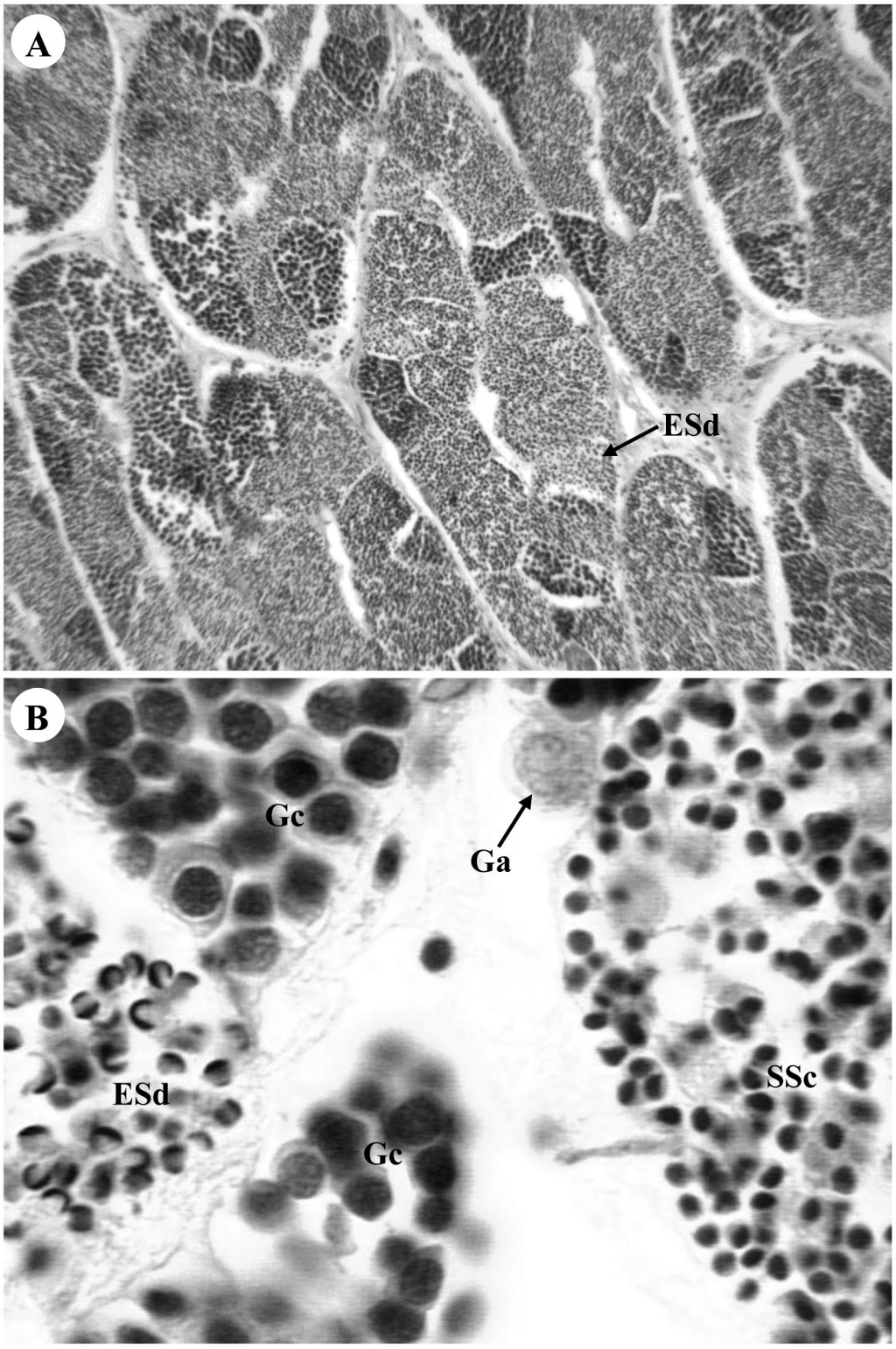

Figure 6

Phase VII testes (early spermatid phase) of a 326 mm *Sebastes elongatus* with primary and tertiary spermatogonia, secondary spermatocytes, and early spermatids. Collected 23 September 1995. (A) 235× and (B) 2000×.

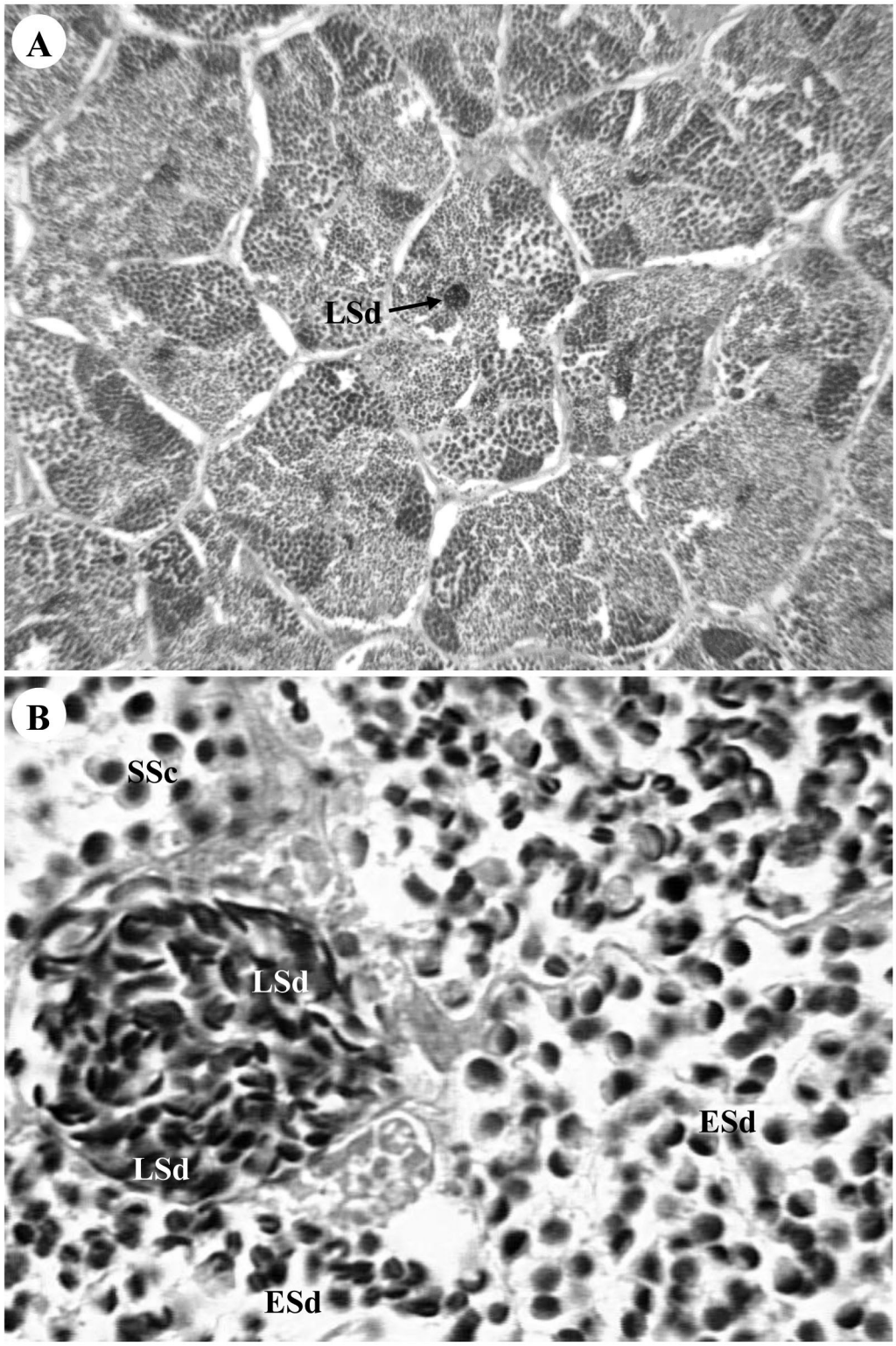

Figure 7

Phase VIII (late spermatid phase) of a 219 mm *Sebastes elongatus* with secondary spermatocytes, and early and late spermatids. Collected 23 November 1995. (A) 235× and (B) 2000×.

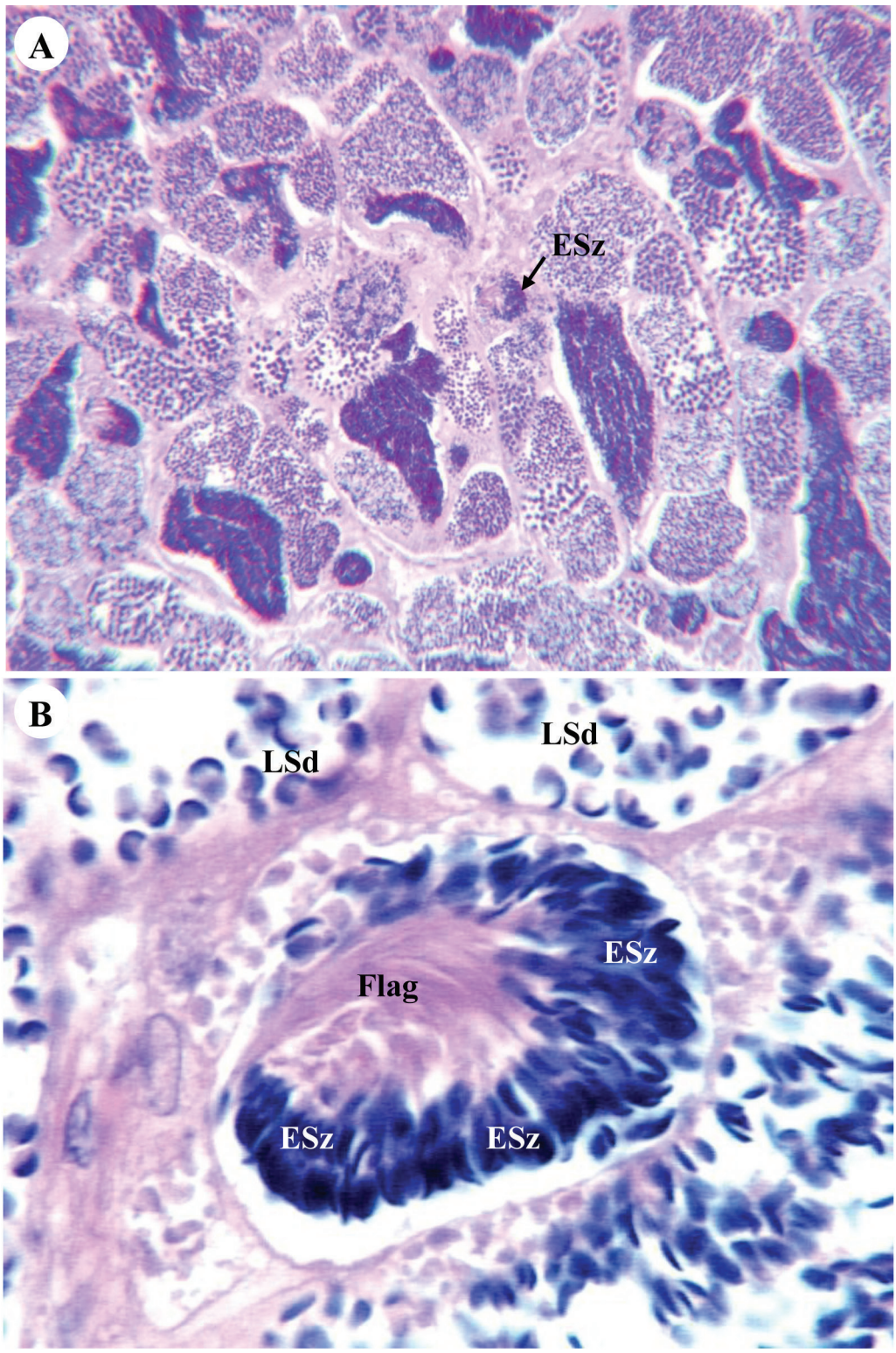

Figure 8

Phase IX testes (early spermatozoa phase) of a 294 mm *Sebastes elongatus* with late spermatids and early spermatozoa. Collected 16 December 1995. (A) 235× and (B) 2000×.

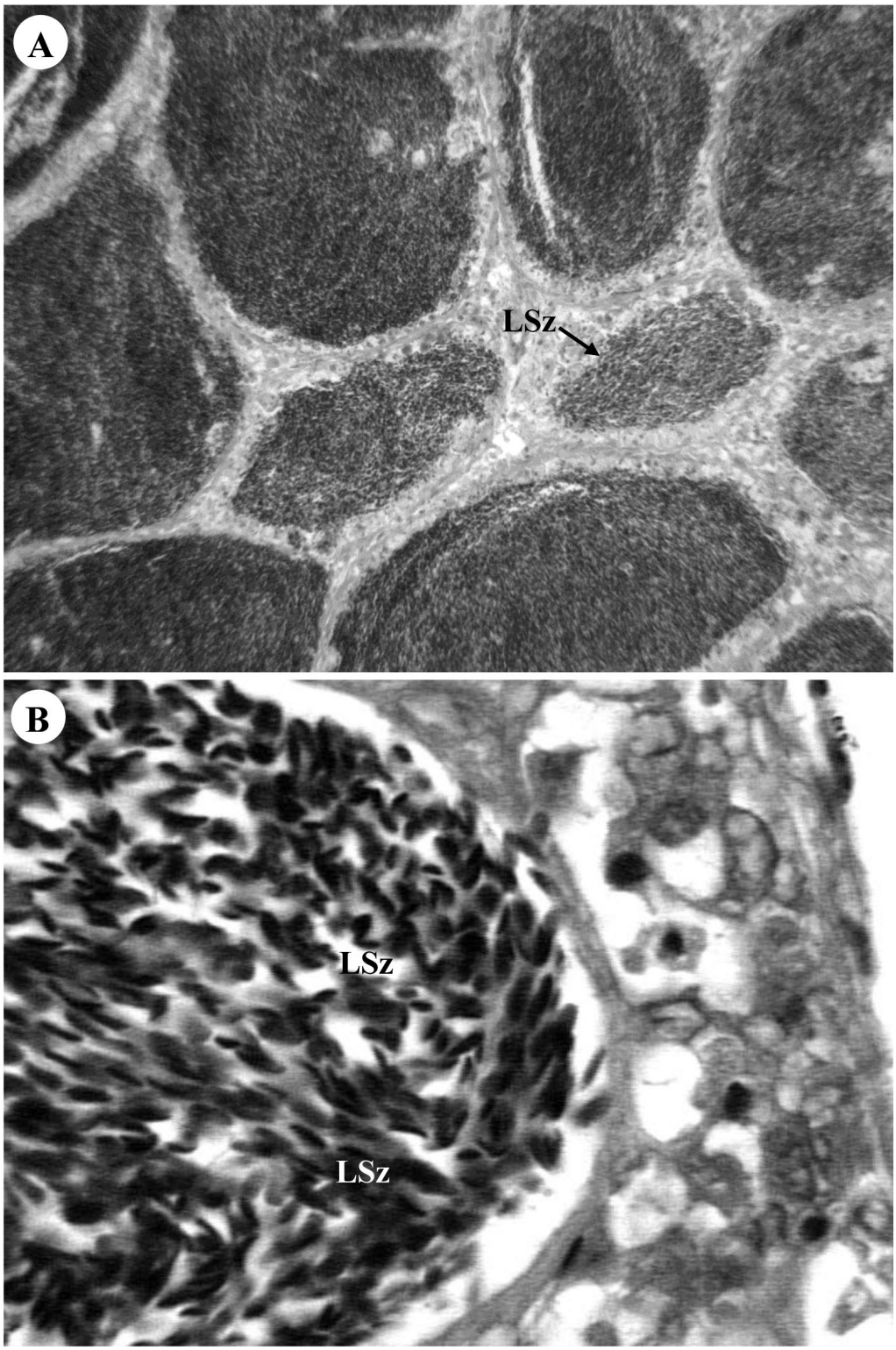

Figure 9

Phase X testes (late spermatozoa phase) of a 278 mm *Sebastes elongatus* with late spermatozoa. Collected 28 February 1995. (A) 235× and (B) 2000×.

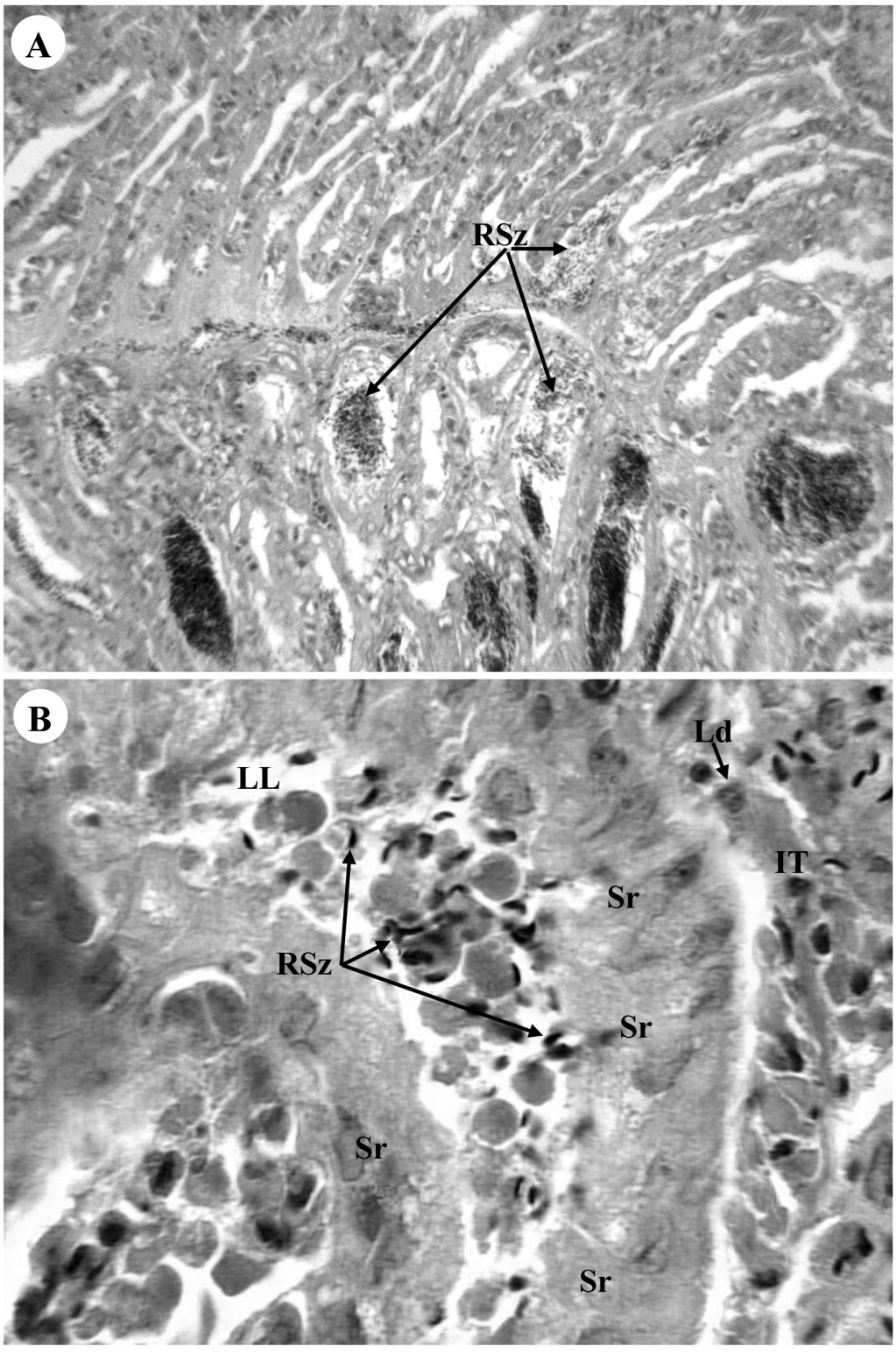

Figure 10

Early phase XI testes (residual spermatozoa phase) of a 338 mm *Sebastes elongatus* with residual spermatozoa and primary spermatogonia. Collected 20 April 1995. (A) 235× and (B) 2000×.

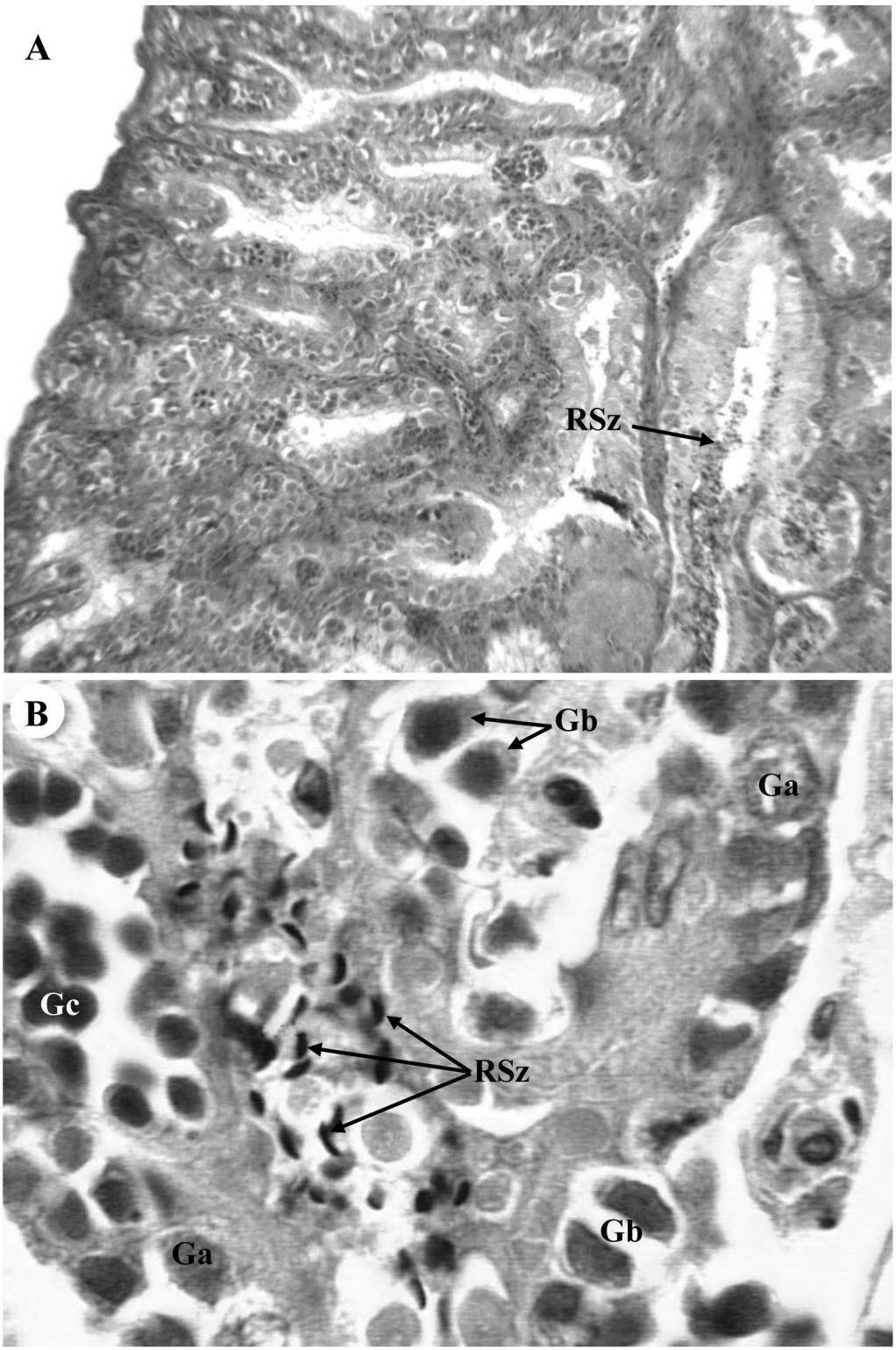

Figure 11

Late phase XI testes (residual spermatozoa phase) of a 237 mm *Sebastes elongatus* with residual spermatozoa, and primary, secondary, and tertiary spermatogonia. Collected 10 June 1995. (A) 235× and (B) 2000×.

Ovary development

The *Sebastes* ovary is attached dorsally and is a paired, elongate structure suspended in the posterior portion of the peritoneal cavity. Each lobe of the ovary is a thin-walled, sac-like structure that is tapered posteriorly and fuses with the other lobe to form a single oviduct. The oviduct extends ventrally between the urinary tract and intestine; the most posterior portion forms the genital opening which is located posterior to the anus and anterior to the urinary opening (Moser, 1967a).

Oogenesis is the transformation of oogonial cells into oocytes through a series of cell divisions, with associated cytoplasmic and chromosomal changes. In the adult ovary, oogonia continue to divide and multiply by ordinary mitosis. Oocytes are formed from oogonia via meiosis which is arrested at the first prophase and second metaphase stages. The development of the oocytes was divided into ten stages based on the scheme first used by Yamamoto (1956) for the flounder (*Liopsetta obscura*), with one additional stage and other minor modifications for use in the viviparous Sebastes. Figures 12–14 are presented to illustrate comparative sizes of developing oocytes. Specific oocyte features as discussed in relation to Figures 12–14 and associated with ovarian development are depicted in Figs. 15–24.

Oogonia

Stage 0: oogonia (8–10 µm) (Fig. 12)

These are very small, round germ cells still capable of mitotic division and usually found in nests in the epithelium of the ovarian lamellae. Each oogonium has a thin layer of clear cytoplasm and a relatively large nucleus, which stains lightly with H&E. In the center of the nucleus is a single large nucleolus.

First growth interval

Stage i: chromatin nucleolus (10–20 µm) (Fig. 12)

These are small oocytes with a nucleus now surrounded by a thin layer of weakly staining (blue) cytoplasm. The nucleus still contains a single large nucleolus, but is now encircled by prominent chromatin threads.

Stage ii: early perinucleus (25–70 µm) (Fig. 12)

Oocyte size increases as the nucleus grows and the cytoplasm thickens. From this point forward the enlarging nucleus is sometimes referred to as the "germinal vesicle." The cytoplasm is basophilic, staining a very dark blue, and is uniform. Many oocytes at this stage are polyhedral in shape. There is one relatively large nucleolus with several smaller nucleoli developing around the periphery of the nucleus. A thin layer of follicle cells begins to form around the oocyte against the zona radiata.

Stage iii: late perinucleus (60–130 µm) (Fig. 12)

The oocyte continues to grow in diameter and becomes more rounded. The cytoplasm is still basophilic but does not stain as deeply and is sometimes zoned (peripherally located as a result of yolk accumulation). The nucleoli become more numerous and uniform in size along the periphery of the nucleus, and lampbrush chromosomes become apparent. This is the end of meiotic prophase and the most advanced stage of oocyte growth that is present throughout the year. Oocytes in this stage are sometimes termed "resting oocytes." The chromosomes of the resting oocytes become arrested at late diplotene of the first meiotic division (Tokarz, 1978). The Balbiani body or yolk nucleus, which contains a number of cytoplasmic organelles including mitochondria, Golgi bodies, and endoplasmic reticulum, (Guaraya, 1979) is formed at this stage.

Second growth interval

Stage iv: oil vacuole (110–190 µm) (Fig. 13)

In the second growth interval, the shape of the oocyte is more spherical. Transparent oil vacuoles begin to form in the perinuclear cytoplasm. In the nucleus, lampbrush chromosomes are often visible (see Fig. 13-iv). The follicle is thickened and is composed of a layer of nucleated cells (granulosa) and a thin layer of connective tissue to the exterior (see Fig. 17). Most other teleosts would start forming cortical alveoli (or yolk vesicles) in the cortical cytoplasm at this stage but they are notably lacking or at least much less prominent in *Sebastes*. Hence, we propose the new name "oil vacuole" for this stage in *Sebastes*, which is equivalent to the "yolk vesicle stage" in Yamamoto (1956).

Stage v: 1° yolk (165–240 µm) (Fig. 13)

Vitellogenesis begins at this stage with the appearance of eosinophilic yolk granules in the cortical cytoplasm (see Fig. 19). As this stage progresses, the yolk granules begin to move centripetally from the periphery of the cytoplasm and the oil vacuoles move centrifugally from the perinuclear cytoplasm.

Stage vi: 2° yolk (200–265 µm) (Fig. 13)

At this stage the yolk granules increase in number and coalesce into yolk globules. The oil vacuoles also increase in size and number. As both the yolk droplets and oil vacuoles continue to proliferate, they fill their respective spaces in the cytoplasm until they meet and form two layers (or rings) against each other.

Stage vii: 3° yolk (230–350 µm) (Fig. 13)

Oil vacuoles and yolk globules are no longer segregated and completely fill the cytoplasm in a randomly dispersed pattern. The nonstaining oil vacuoles are much larger than the variably, eosinophilic stained yolk globules. The zona radiata has increased in thickness to 5–7 µm.

Oocyte maturation interval

Stage viii: migratory nucleus (325–670 µm) (Fig. 14)

Meiosis resumes at this stage as the nucleus moves from the center of the cell towards the periphery of the cell and the micropyle. The oil vacuole begins to coalesce into a large vacuole, occupying the area that the nucleus has vacated. The follicle has stretched and thinned due to the rapid growth of the oocytes. Lampbrush chromosomes are visible at this stage.

Stage ix: prematuration (440–710 µm) (Fig. 14)

The nuclear membrane dissolves and the nucleus begins to disappear, more oil coalesces from the vacuoles into the central oil vacuole, and the yolk globules begin to coalesce into a yolk mass surrounding the oil vacuole. The walls of the granulosa cells disappear, leaving a single cell with many nuclei or a syncytial arrangement.

Stage x: mature (540–725 µm) (Fig. 14)

When the nucleus has disappeared, the oocyte can be classified as mature (Wasserman and Smith, 1978). The yolk mass (now completely coalesced and transparent pink in H&E stain) surrounds the oil vacuole which is near the periphery of the cell and clear in color.

Ovary phases

Twelve histological development phases closely parallel the developmental stages of individual oocytes. Each phase is defined as beginning with the first appearance of the same stage oocyte and ending with the first appearance of the next stage oocyte in the ovary as a whole. The ovary development phases are followed by embryonic development and atresia (or spent) phases. The various phases are illustrated in Figs. 15–24 with sections from *S. proriger* ovaries.

Phase I: chromatin nucleoli and Phase II: early perinucleus

Ovaries from fish as small as 120 mm were collected and all had some oocytes that had developed past the early perinucleus stage. Thus, no ovary with less development than the late perinucleus phase (III) was encountered.

Phase III: late perinucleus (Fig. 15)

Ovaries in this phase contain oogonia, chromatin nucleoli, early perinucleus, and late perinucleus oocytes. The oogonia and chromatin nucleoli stages are found in oogonial nests. As the late perinucleus stage oocytes grow, they are found less clustered, with space for substantial growth during vitellogenesis. Spawning has occurred previously in the ovary in Fig. 15, as evidenced by the remnants of a post-ovulatory follicle.

Phase IV: oil vacuole (Fig. 16)

The oil vacuole phase has all the oocyte stages of the previous phase plus the oil vacuole stage. The growing oocytes are beginning to grow synchronously, but the oogonial nests are still prominent.

Phase V: 1° yolk (Fig. 17)

Primary yolk phase ovaries may include all the oocyte stages of the previous phases plus the primary yolk stage signaling the onset of vitellogenesis. Spermatozoa are stored in the interstitial tissue of a primary yolk phase ovary awaiting the maturation of the oocyte. At this point, the spermatozoa have lost their flagella.

Phase VI: 2° yolk (Fig. 18)

In this ovary phase two distinct modes of oocytes have begun to emerge. The mode in first growth phase (Og-LPN) is arrested at the late perinucleus stage and is sometimes called the "reserve fund," while the mode at the secondary yolk stage progresses in a synchronized manner through the second growth phase (vitellogenesis).

Phase VII: 3° yolk (Fig. 19)

This ovary phase, like the previous one, has two modes of oocyte development. The first is still the first growth phase oocytes, which occasionally include an oil vacuole stage oocyte. The second mode has now progressed to the tertiary yolk stage.

Phase VIII: migratory nucleus (Fig. 20)

The second mode has now progressed to the migratory nucleus stage oocyte. The nuclear membrane has disappeared. An occasional atretic vitellogenic oocyte is seen.

Phase IX: prematuration (Fig. 21)

The first growth stage of oocytes is still found in this ovarian phase but is now dwarfed by the increasing size of prematuration oocytes.

Phase X: mature (Fig. 22)

The interstitial tissue surrounding the mature oocyte is becoming sparse, even though oogonial nests can still be found in the interstitial tissue. Ovulation begins when the oocytes have matured, the stored spermatozoa are released, and fertilization commences.

Table 3

Summary of the gross and histological phases and cell stages of ovaries in *Sebastes*. Table partially reproduced with permission from Shaw and Gunderson (2006, 2008).

Gross ovary phase	Histological ovary phase	Oocyte cell stages present
Immature	I,II,III	Og, ON, CN, EPN, LPN
Vitellogenesis	IV, V, VI, VII, VIII, IX	Og, CN, EPN, LPN, OV, PY, SY, TY, MN, PM
Clear egg	X,XI	Og, CN, EPN, LPN, MO, Em, *
Eyed larvae	XI	Og, CN, EPN, LPN, Em, *
Spent	XII	REm, EF, Og, CN, EPN, LPN, OV, *
Regenerating	II, III	REm, Og, ON, *

*Atresia is potentially possible in all phases of ovarian development. In this study, atresia was rare as reported by de Bruin et al. (2004) and was infrequently encountered during Clear egg, Eyed larvae, Spent, and Regenerating ovarian phases.

Phase XI: embryonic development (Fig. 23)

Embryos develop synchronously through the 33 embryonic stages within the ovary and hatch before parturition. Embryos in Fig. 23 are at stage 33 (hatched, preborn larvae).

Phase XII: atresia (Fig. 24)

Oocytes that have failed to mature and residual embryos, which remain unborn after parturition has ceased, are found in various atretic states at this phase. Postovulatory follicles that have not yet collapsed and are undergoing atresia are seen in individuals that have recently undergone parturition.

Gross morphological phases of the ovary

Immature: Ovaries are small and no oocytes are visible. The color is translucent pink and no dark spots or blotches are present.

Vitellogenesis: Ovaries are enlarging and are opaque pink to yellow in color. The small oocytes are now discernible and opaque.

Clear egg: The eggs are now mature, large, translucent, and yellow. Ovulation and fertilization occur early in this stage so most eggs in this stage have been fertilized. The ovary wall is thin and flaccid.

Eyed larvae: Black pigmentation in the larvae eyes impart a grey color to the ovary which is less translucent than the previous stage. The ovary wall is fragile.

Spent: The ovary is flaccid and nearly empty. Residual larvae are often present and are seen as dark spots against a purple background.

Regenerating: Few residual larvae may be present. Organ is reduced in size but undergoing reorganization of muscle, blood vessels, and ovarian wall. Becoming firm in comparison to a spent organ.

As previously noted for the testis, field visual examination of ovary maturity provides an on-site, but potentially inaccurate, diagnosis of gonad development. Histological or microscopic analysis provides a more accurate and complete description of ovarian development, but is not as rapid. Comparison of the two methods is provided in Table 3 which summarizes tissue and cellular features associated with visually described ovary development.

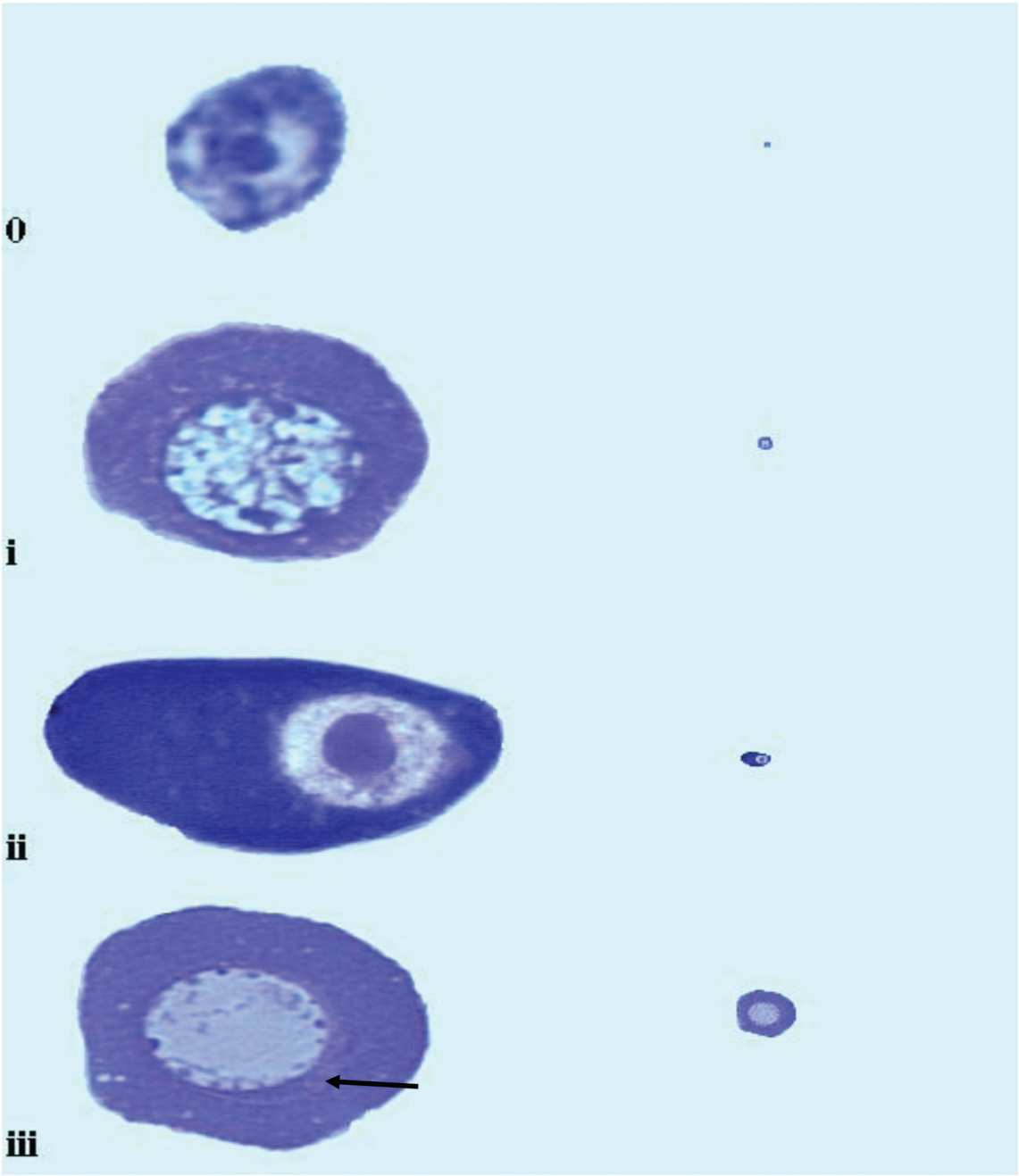

Figure 12

Oogonium and first growth interval oocytes. Images on the left are standardized to about the same size for all stages. Images on the right are magnified to the same power (50×) to show relative sizes. Stages shown are (0) oogonium, (i) chromatin nucleolus, (ii) early perinucleolus, and (iii) late perinucleolus with Balbiani body (arrows).

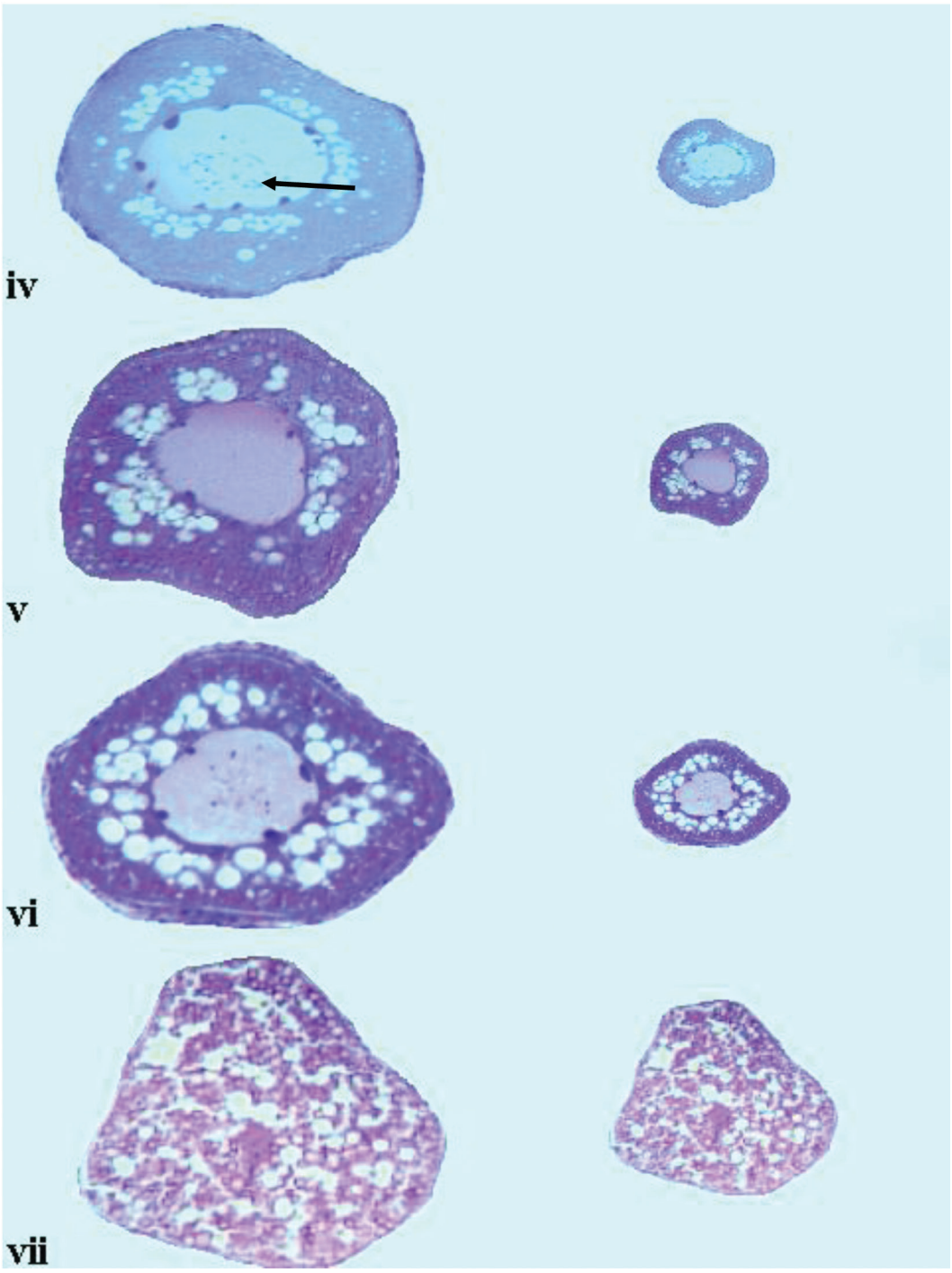

Figure 13

Second growth interval oocytes. The images on the left are standardized to about the same size for all stages. The images on the right are magnified to the same power (50×). Stages shown are (iv) oil vacuole with lampbrush chromosomes (arrows), (v) 1° yolk, (vi) 2° yolk, and (vii) 3° yolk.

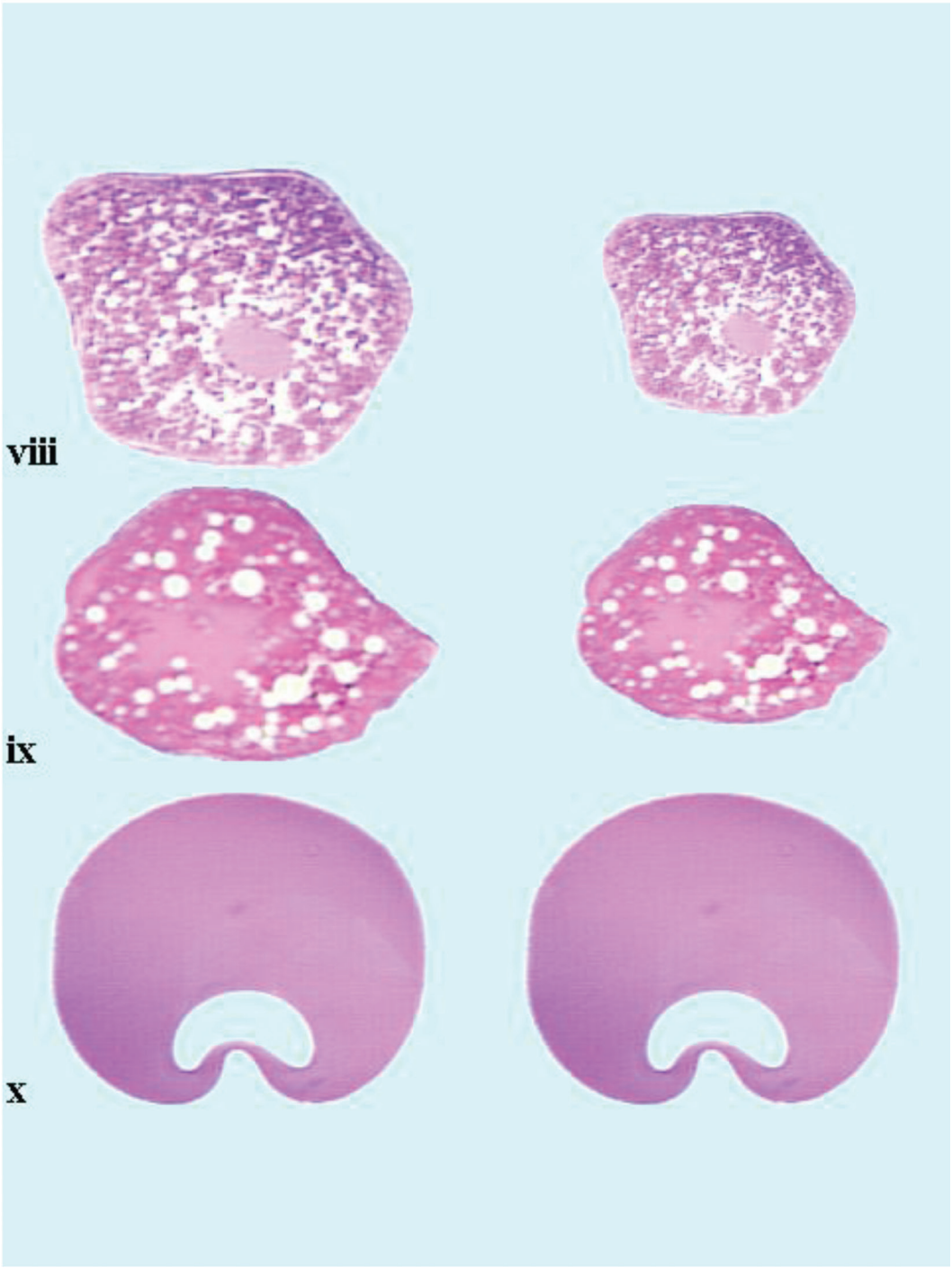

Figure 14

Maturation interval oocytes. The images on the left are standardized to about the same size for all stages. The images on the right are magnified to the same power (50×). Stages shown are (viii) migratory nucleus, (ix) prematuration, and (x) mature.

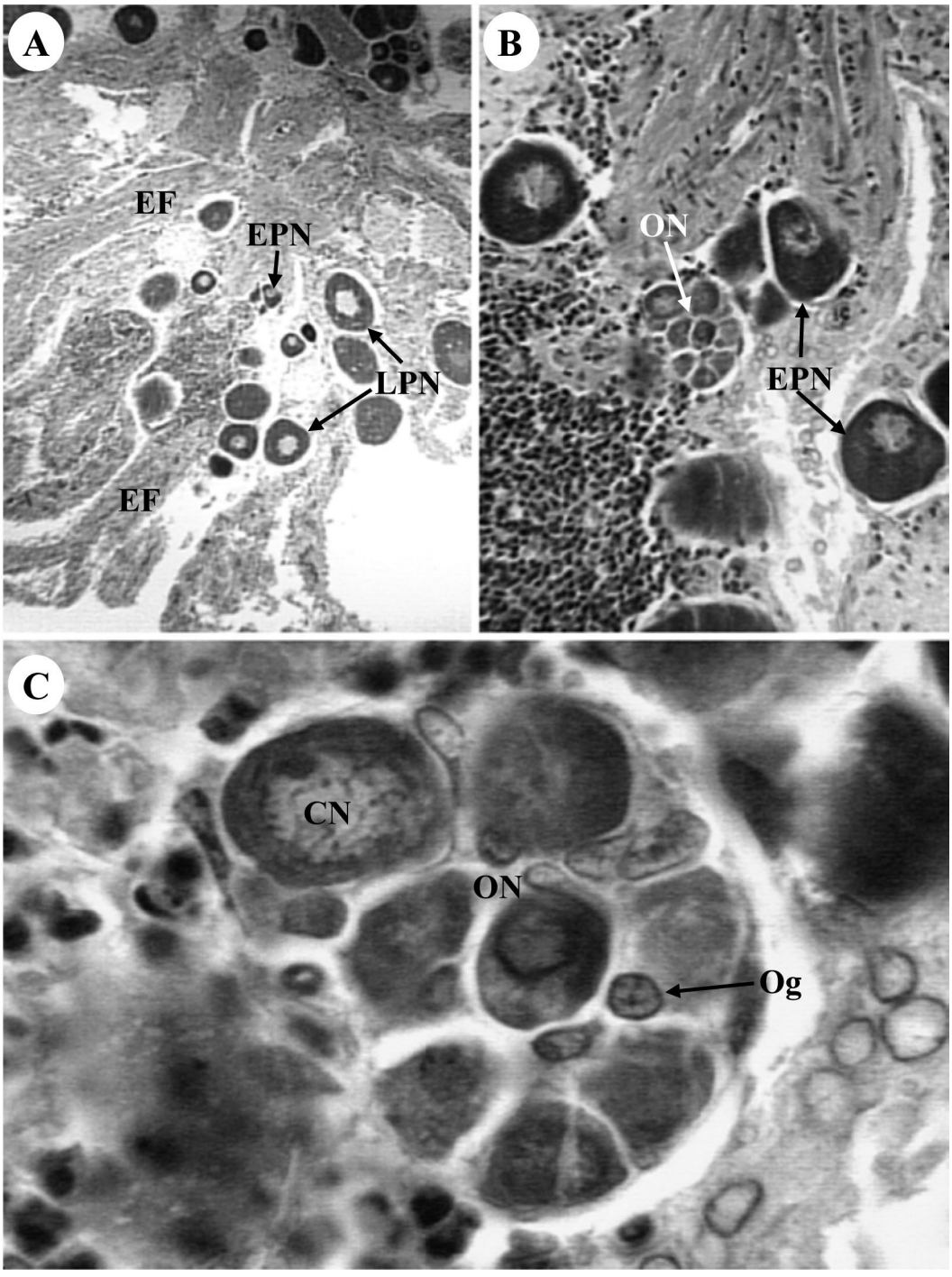

Figure 15

Phase III ovary (late perinucleus phase) of a 320 mm *Sebastes proriger* with oogonia, chromatin nucleolus, early perinucleus, and late perinucleus stage oocytes. Collected 18 August 1995. (A) 100×, (B) 400×, and (C) 2000×.

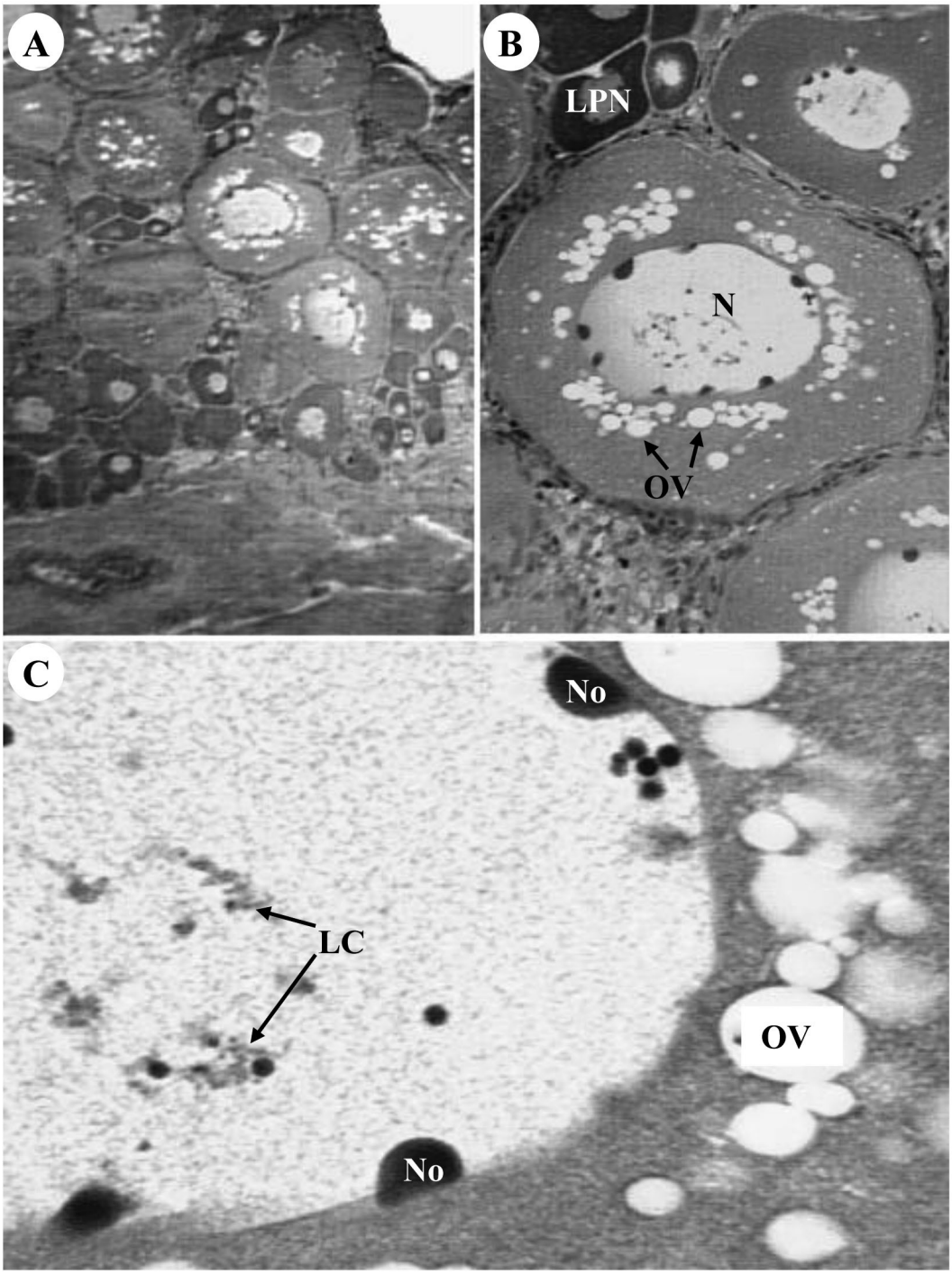

Figure 16

Phase IV ovary (oil vacuole phase) of a 318 mm *Sebastes proriger* with late perinucleus and oil vacuole stage oocytes. Collected 23 September 1995. (A) 100×, (B) 400×, and (C) 2000×.

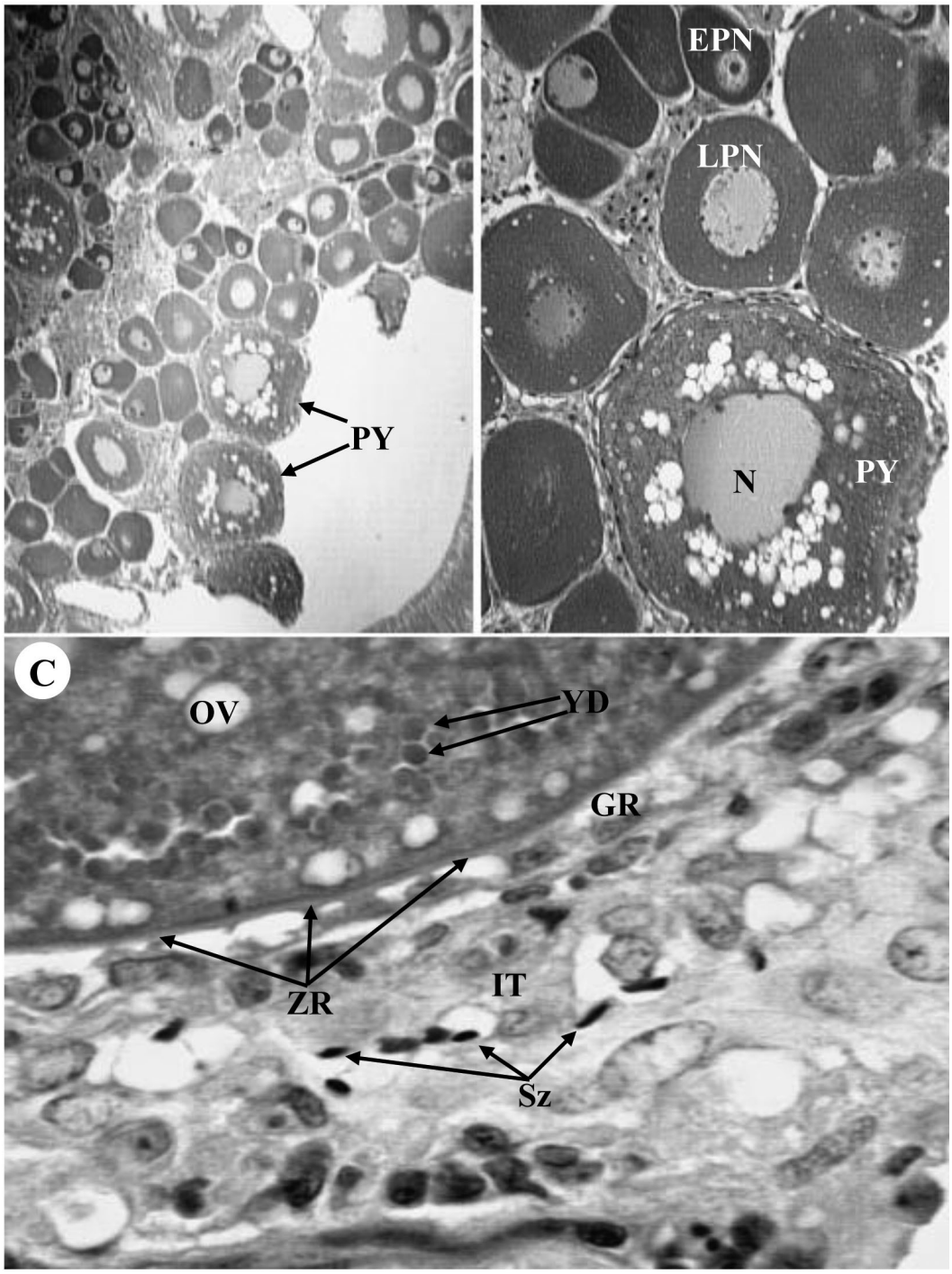

Figure 17

Phase V ovary (primary yolk phase) of a 325 mm *Sebastes proriger* with early perinucleus, late perinucleus, and 1° yolk stage oocytes. Stored spermatozoa without flagella are also present in the interstitial tissue. Collected 23 November 1995. (A) 100×, (B) 400×, and (C) 2000×.

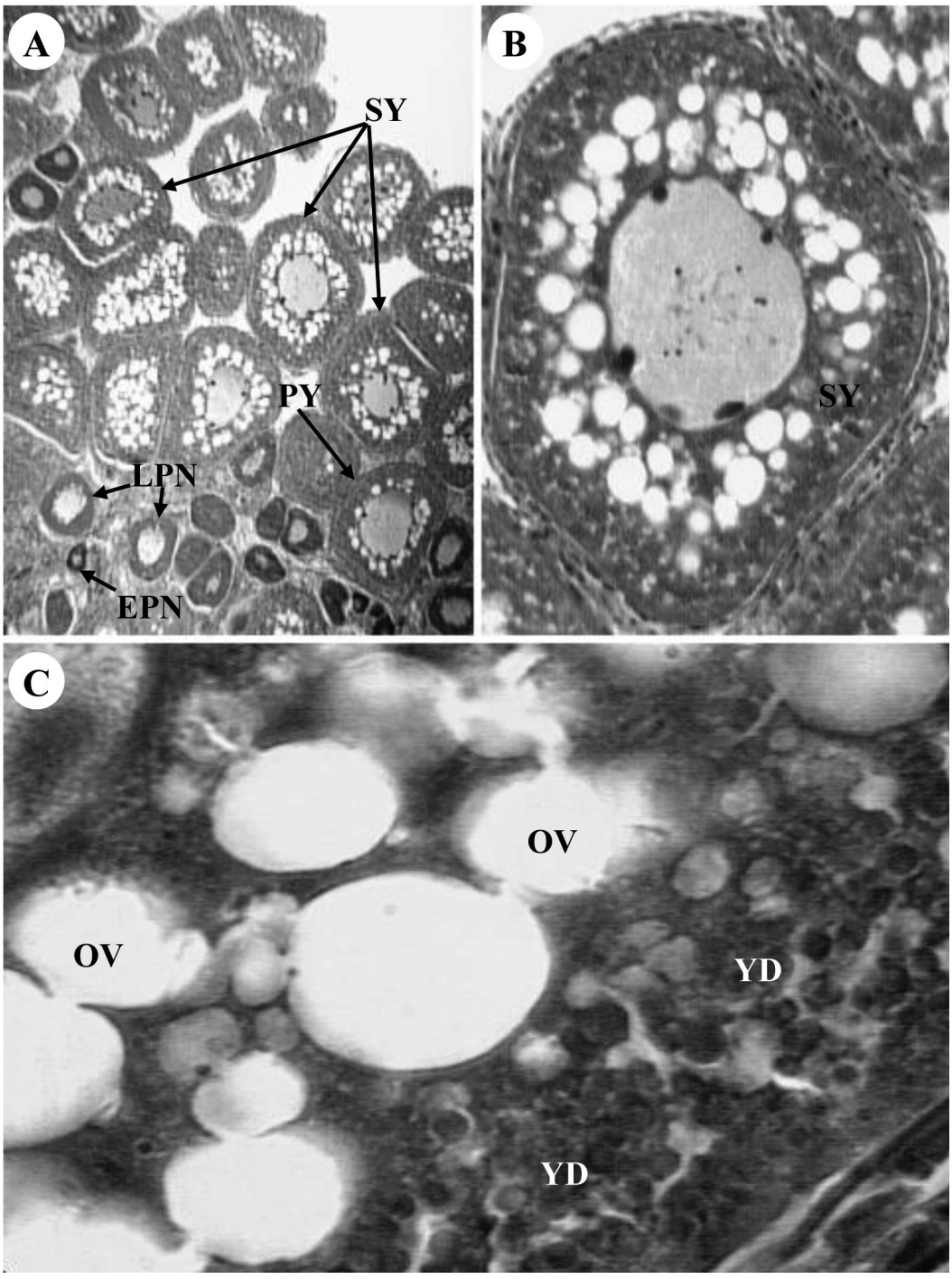

Figure 18

Phase VI ovary (secondary yolk phase) of a 314 mm *Sebastes proriger* with early perinucleus, late perinucleus, 1° yolk, and 2° yolk stage oocytes. Collected 23 November 1995. (A) 100×, (B) 400×, and (C) 2000×.

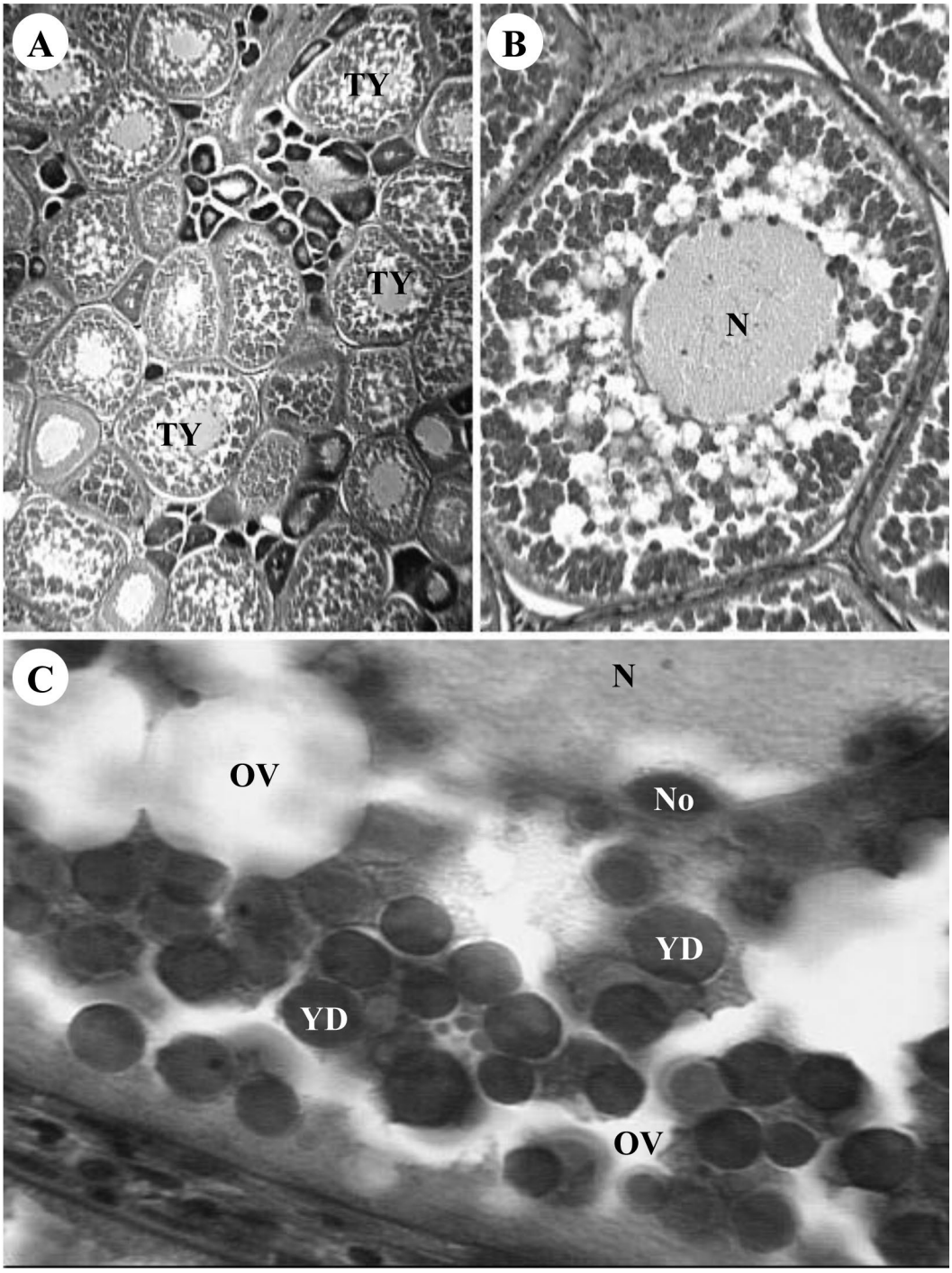

Figure 19

Phase VII ovary (tertiary yolk phase) of a 328 mm *Sebastes proriger* with early perinucleus, late perinucleus, oil vacuole, and 3° yolk stage oocytes. Collected 16 December 1995. (A) 100×, (B) 400×, and (C) 2000×.

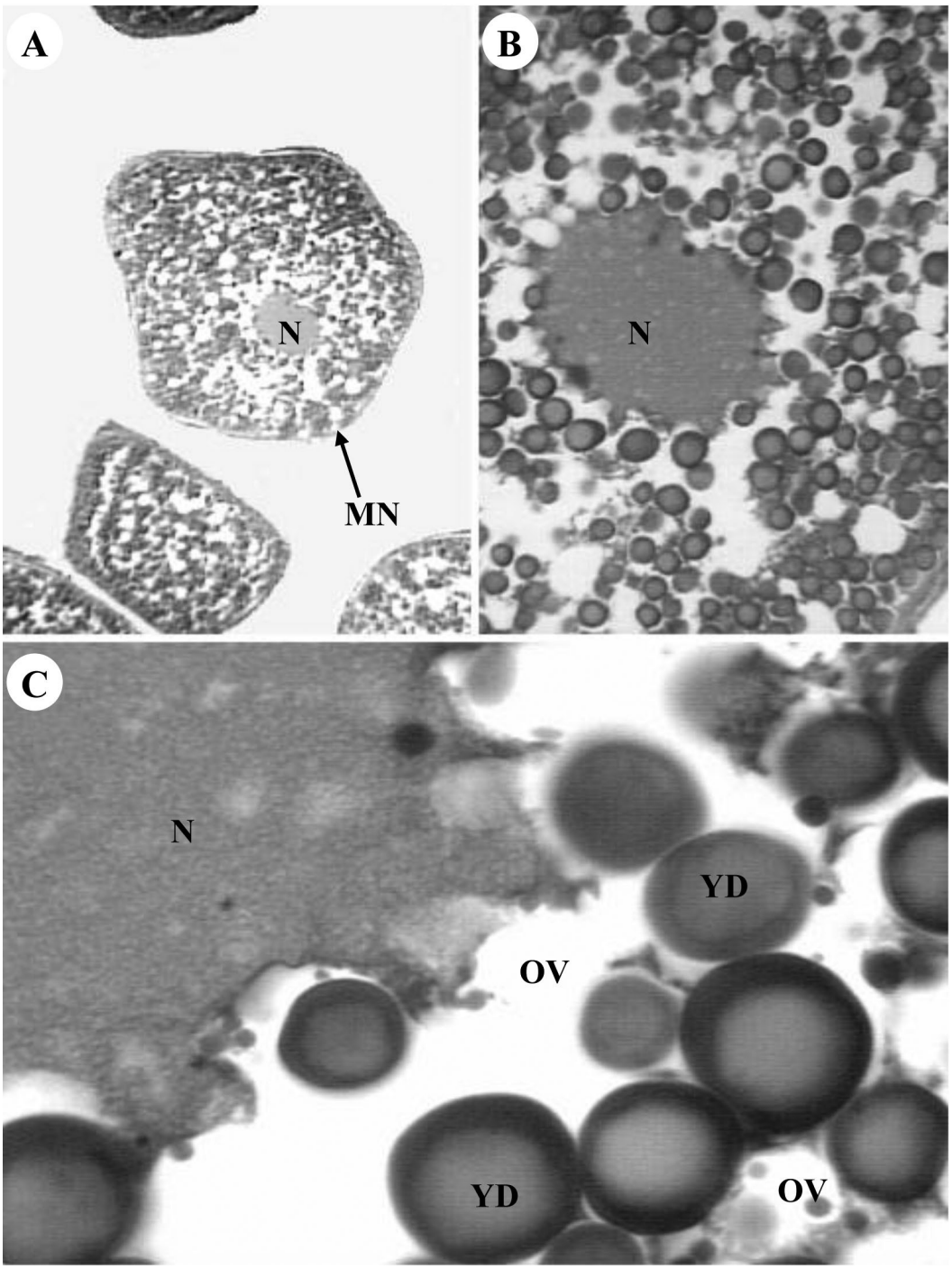

Figure 20

Phase VIII ovary (migratory nucleus phase) of a 354 mm *Sebastes proriger* with migratory nucleus stage oocytes. Collected 28 February 1995. (A) 100×, (B) 400×, and (C) 2000×.

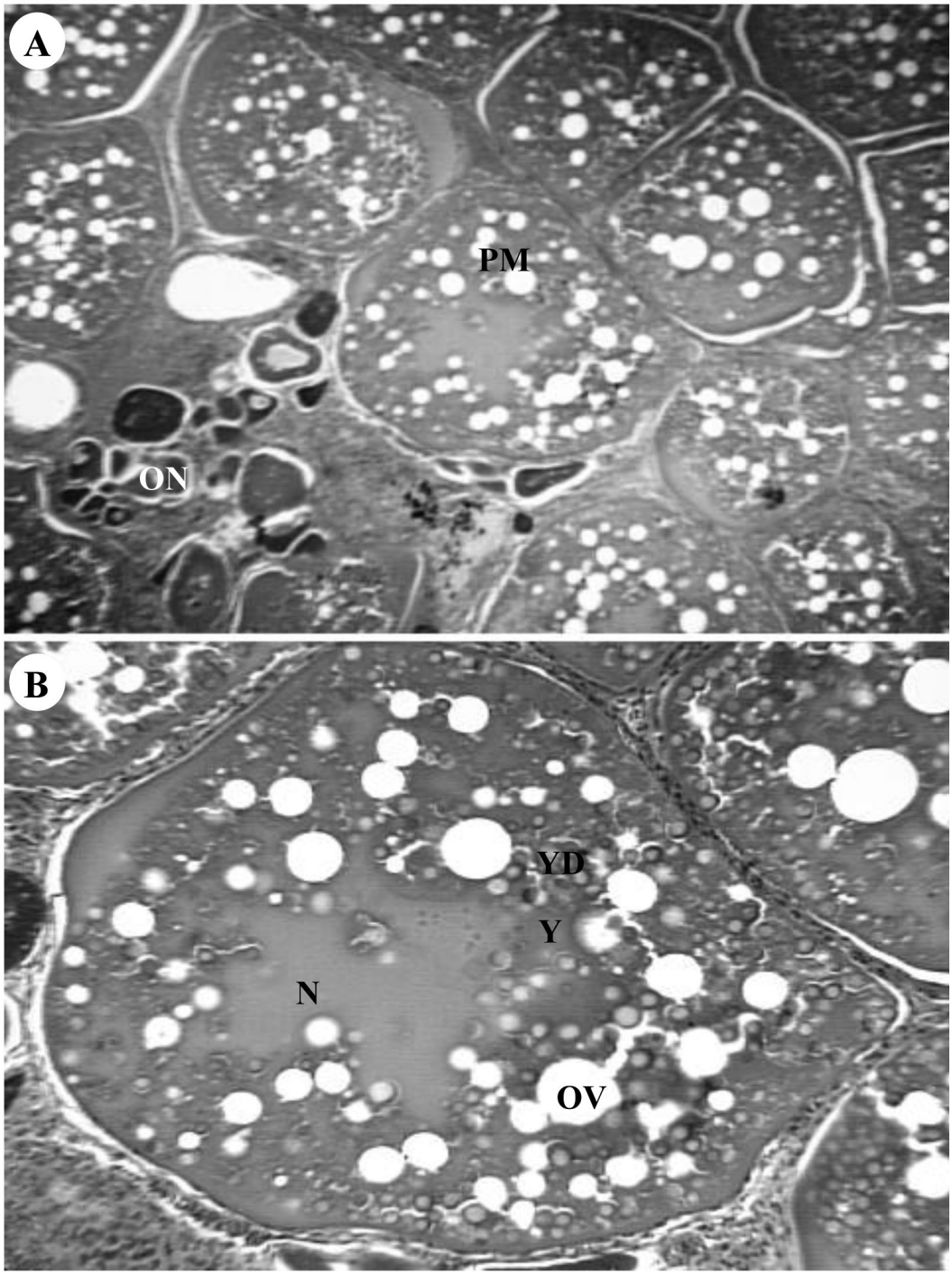

Figure 21

Phase IX ovary (prematuration phase) of a 300 mm *Sebastes proriger* with early perinucleus, late perinucleus, oil vacuole, and prematuration nucleus stage oocytes. Collected 20 April 1995. (A) 100× and (B) 200×.

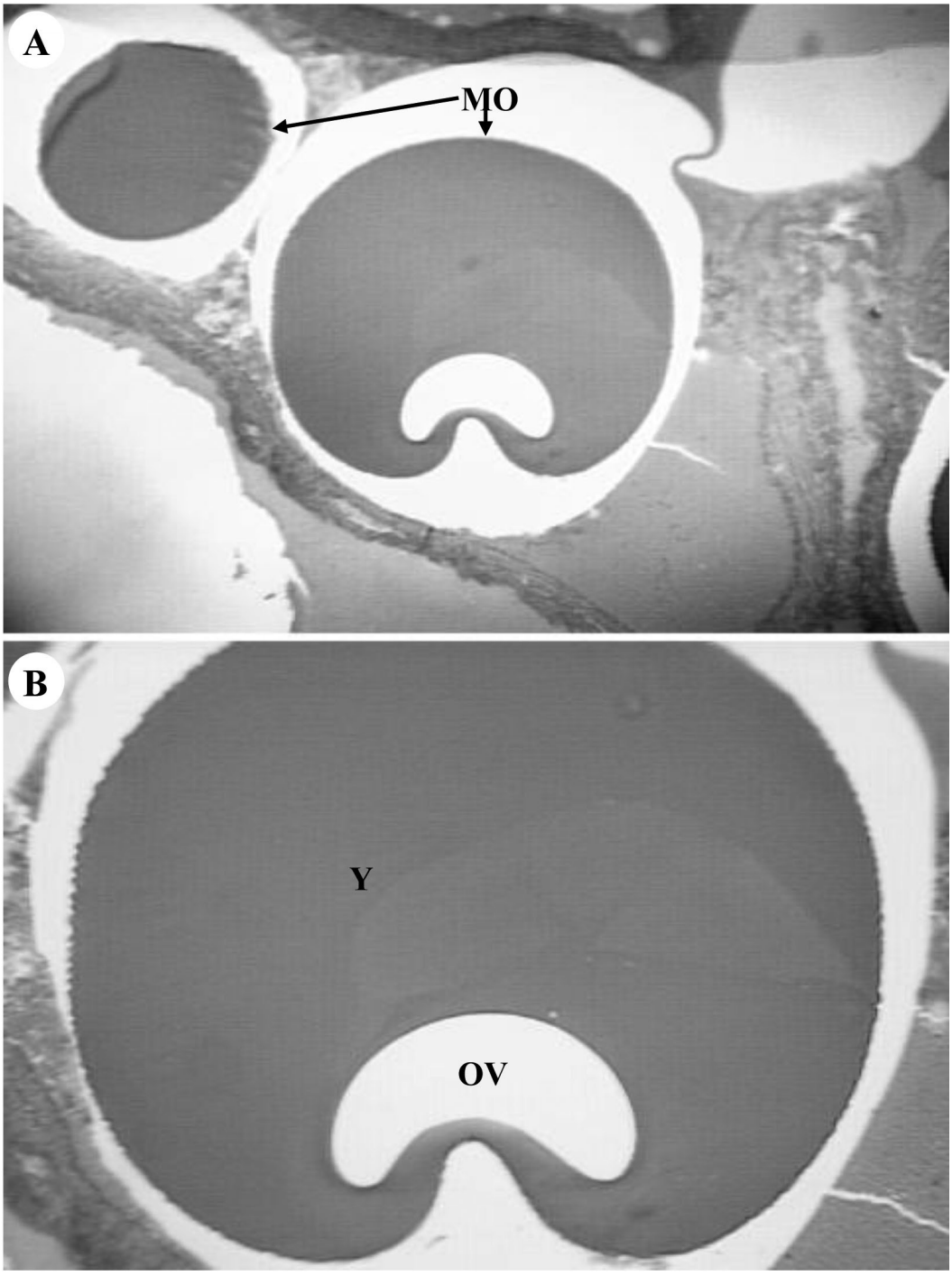

Figure 22

Phase X ovary (mature phase) of a 322 mm *Sebastes proriger* with mature stage oocytes. Collected 9 May 1995. (A) 100× and (B) 200×.

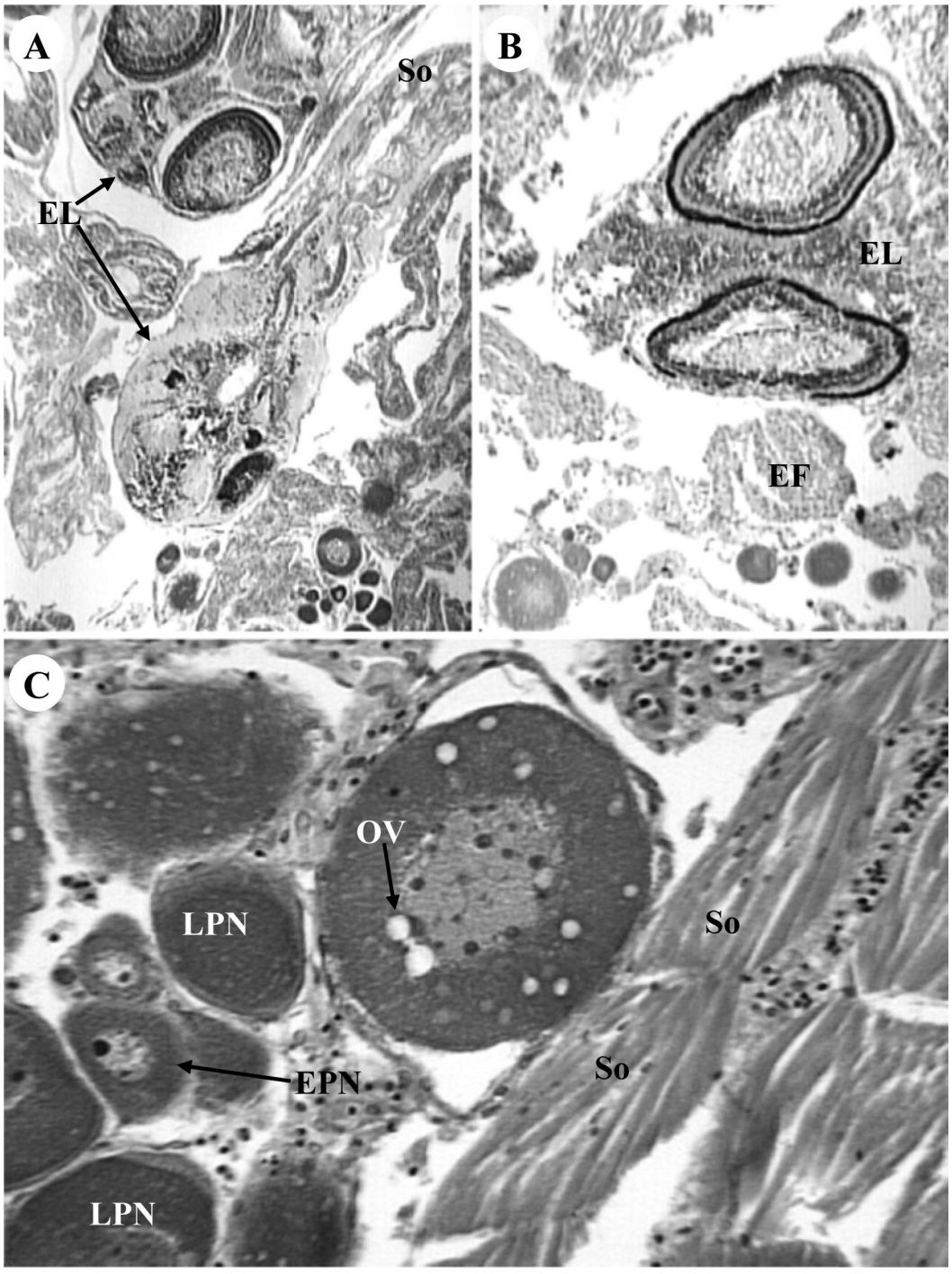

Figure 23

Phase XI ovary (embryonic phase) of a 345 mm *Sebastes proriger* with early perinucleus, late perinucleus, oil vacuole (early), eyed larvae, and empty follicles stages. Collected 10 June 1995. (A) 50×, (B) 100×, and (C) 400×.

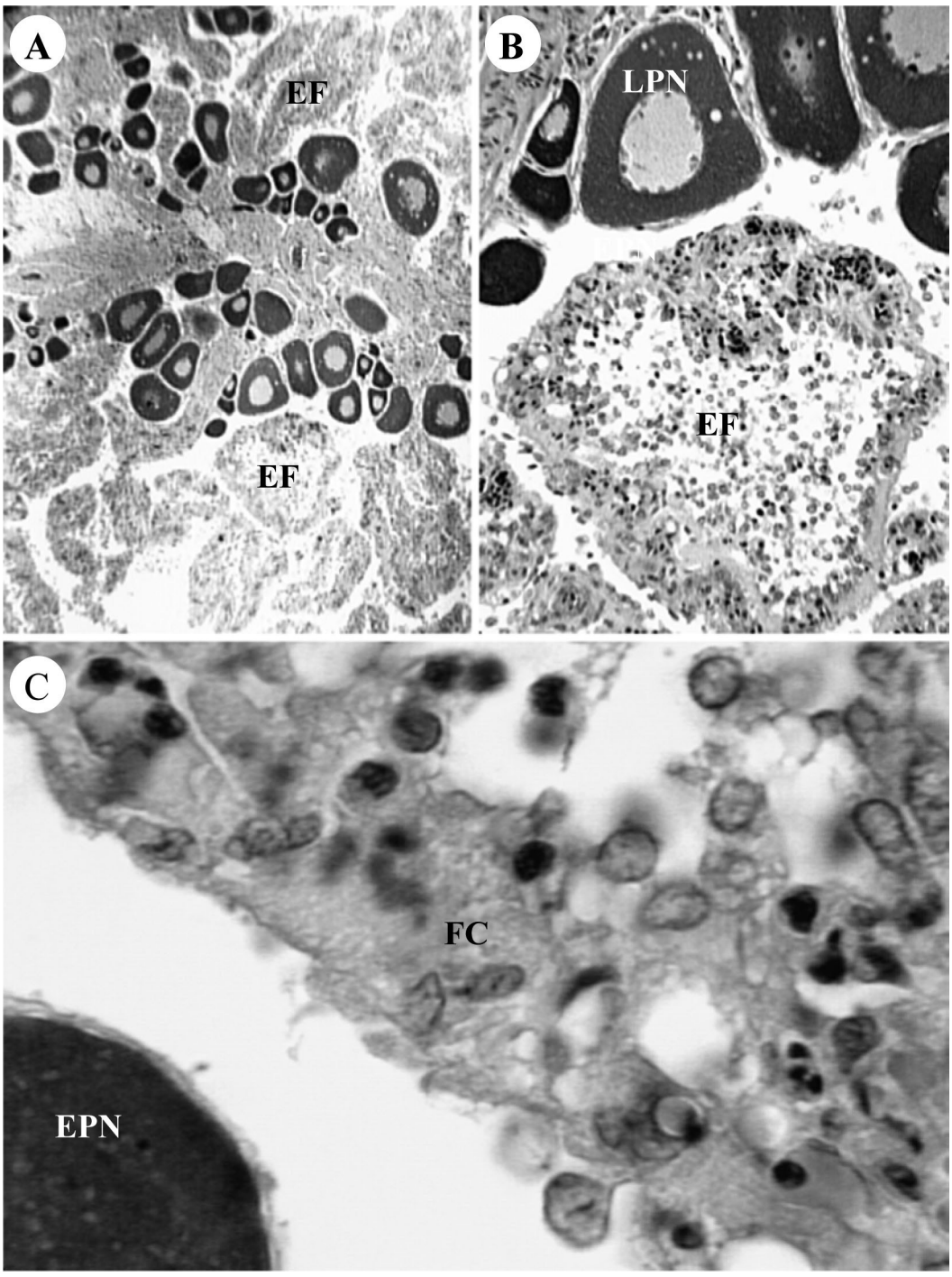

Figure 24

Phase XIII ovary (atresia phase) of a 323 mm *Sebastes proriger* with early perinucleolus, late perinucleolus, and empty follicles stages. Collected 15 July 1995. (A) 100×, (B) 400×, and (C) 2000×.

Embryonic development

The normal embryonic development for *S. schlegeli* (N=33 stages) has been briefly described by Yamada and Kusakari (1991) following the embryonic model of Oppenheimer (1937) for *Fundulus heteroclitus* (N=34 stages). Their description was based on a series of embryos gently suctioned from live females every 12 to 15 days and examined macroscopically. The extracted tembryos were placed in saline solution in a petri dish and then observed and photographed under a dissecting microscope. Some early stage embryos were followed over time in the petri dish. In contrast, more comprehensive time-related accounts of normal embryonic stages are available for the zebrafish, *Danio rerio* (Kimmel et al., 1995) and medaka, *Oryzias latipes* (Iwamatsu, 2011). In both instances, the time after fertilization is correlated with developmental stage and both provide "fill-in" observations and terms of reference in our description of rockfish embryonic development.

Embryonic development of the *Sebastes* species in this histological study closely tracks embryonic development as briefly described by Yamada and Kusakari (1991) for *S. schlegeli*, and which is supported by general observations on embryo development in yellowtail rockfish (*S. flavidus*, Bowers 1992), and grass (*S. rastrelliger*) and brown (*S. auriculatus*) rockfish (see Table 4; Chaillé, 2006). In general, embryonic descriptions have previously been performed almost exclusively by macroscopic examination. Histological and ultrastructural descriptions are uncommon and rarely supported by parallel macroscopic studies. Bowers (1992) provided an abbreviated microscopic analysis on field collected specimens. Our study is solely dependent upon examination of histological sections that visually represented gross morphological phases of the ovary. Because our study is 2-dimensional as opposed to traditional 3-dimensional macroscopic analyses, not all stage features were observed in our study or were not observed at the specific stages noted by Yamada and Kusakari (1991). As a result, figures were occasionally selected that demonstrated representative organ development for approximate aged embryos (see Table 4). Regardless, our observations did appear to closely parallel the observations of Yamada and Kusakari (1991), but we also recommend reviewing the manuscripts by Kimmel et al. (1995) and Iwamatsu (2011) for detailed fish embryology.

Stage x: mature (540–725 µm) (Fig. 14x)

When the nucleus has disappeared, the oocyte can be classified as mature (Wasserman and Smith, 1978). The yolk mass (now completely coalesced and transparent pink in H&E stain) surrounds the oil vacuole which is near the periphery of the cell and clear in color.

Stage 1: mature ovum (Fig. 25)

An eosinophilic yolk (Y) mass makes up the majority of a mature unfertilized ovum, but it also contains a number of oil vacuoles (OV) which are clear and colorless.

Zygote period: a newly fertilized ovum, but prior to cleavage.

Stage 2: formation of blastodisc (Fig. 26)

In stage 2 or the one-cell stage, the fertilized ovum is called a zygote and the blastodisc (Bd) or germinal disc appears on the surface of the yolk.

Cleavage period: a series of cell divisions that occur prior to formation of the blastula. The resulting cells are generally of equal size.

Stage 3: 2-celled embryo (Fig. 27)

The first cleavage yields two cells or blastomeres (Bm), resulting in the 2-celled embryo. The cleavage furrow is oriented along the animal and vegetative poles. This and subsequent collections of blastomeres result in a blastoderm. As division progresses, mitotic figures (MF) may become evident (see Figure 34B).

Stage 4: 4-celled embryo (Fig. 28)

The two blastomeres visible in stage 3 undergo a subsequent cleavage, occurring at right angle to the first cleavage plane and resulting in four basophilic blastomeres easily distinguished from the pink staining eosinophilic yolk (Figure 28C). The four cells are arranged in a 2×2 array. Figure 28B appears to be in transition from a 4-celled embryo to an 8-celled embryo. From this point forward, the number and orientation of the blastomeres is less evident in histological sections. Thus, we rely upon the collective descriptions by Kimmel et al. (1995) and Iwamatsu (2011).

Stage 5: 8-celled embryo (Fig. 29)

A third cleavage plane develops in parallel to the first, dividing the four blastomeres into an 8-celled embryo. The cleavages cut the short axis, resulting in two rows of four cells.

Stage 6: 16-celled embryo (Fig. 30)

The fourth cleavage plane that occurs parallel to the second cleavage results in a 16-celled embryo.

Stage 7: 32-celled embryo (Fig. 31)

The fifth cleavage plane generally divides the marginal 12 blastomeres meridionally into 24. The central four blastomeres divide horizontally into eight blastomeres. During this stage an outer and inner layer are evident in the central region of the blastoderm.

Stage 7 exhibits presumed 32 blastomeres as a result of another cycle of cleavage.

Stage 8: 64-celled embryo (Fig. 32)

After completion of the sixth cycle of cleavages the embryo possesses 64 blastomeres arranged in three layers. This stage has the first appearance of the outer enveloping layer (EVL) composed of the topmost layer of blastomeres of the blastoderm. From this stage forward, cell sizes may vary.

Blastula period: characterized by early metasynchronous cell cycles followed by asynchronous cell cycles, ending with the initiation of epiboly. Cells vary in size.

Stage 9: morula (Fig. 33)

Yamada and Kusakari (1991) note that the morula stage occurs after the 64-cell embryo stage. However, the morula or mulberry stage in animals may occur as early as the 16-cell stage and as late as the 512-cell stage (Iwamatsu, 2011). In fish, the morula is characterized as a dome-shaped aggregate of blastomeres that have not yet started migrating over the yolk of the embryo or progressing into the blastula stages. Cleavage planes vary depending upon the position of the blastomere within the raised, oval, basophilic blastoderm. At about 512 blastomeres, the yolk syncytial layer (YSL) forms (Fig. 36). More current fish development publications abstain from using the term "morula." We continue to use the term as a means of connection to previous literature only.

Stage 10: early blastula (Fig. 34)

The dome-shaped blastoderm is composed of approximately 1000 cells with the inner cells being smaller than the outer blastomeres.

Stage 11: late blastula (Fig. 35)

Blastomeres begin to project into the yolk mass, but the blastoderm is still dome-shaped.

Stage 12: beginning of epiboly (Fig. 36)

The dome shape of the blastoderm begins to flatten as blastomeres migrate over the periphery of the yolk. The yolk syncytial layers (YSL) form earlier, but it is evident in this figure.

Gastrula period: cellular reconstruction of the blastoderm resulting in a three germ-layered structure (organ anlage) and ending with complete epiboly, characterized by embryonic axis/orientation.

Stage 13: early gastrula (Fig. 37)

The blastoderm expands over the surface of the yolk and a thickened margin of the blastoderm yields a presumptive region of the embryonic shield (Fig. 39).

Stage 14: late gastrula (Fig. 38)

The blastoderm covers approximately three-fourths of the yolk sphere.

Segmentation period: somites develop, organogenesis begins, tail appears, and early movement of embryo noted.

Stage 15: embryonic shield (Fig. 39)

The small anlage of stage 13 increases in size; the embryonic shield (ES) is clearly evident at this stage.

Stage 16: head fold (Fig. 40)

The yolk sphere is nearly covered by the blastoderm. The head fold (HF) is a 3-dimensional structure that establishes the anterior end of the developing embryo; the brain, heart, and foregut develop from this anlage. Fig. 40 illustrates stage 16 in transition to stage 17 including development of an optic vesicle (OpV).

Stage 17: optic vesicles (Figs. 40 and 41)

A small optic bud or rudimentary eye first appears on each side of the developing head.

Stage 18: somite formation begins (Figs. 41C and 42)

Derived from the mesoderm; dermis, skeletal muscle, vertebra, and notochord (Noto) formation begins. Somites (So) begin to form and will increase in number as development continues.

Stage 19: fin fold (Fig. 43)

The term refers to shallow membranous fins. The median finfold (FF) is generally the first to appear.

Stage 20: optic cup, 22-23 somites (Fig. 44)

Somites (So) continue to develop. Optic cups (OpC) arise from optic vesicles and are composed of two layers of cells.

Stage 21: auditory placodes (Fig. 45)

Small otic vesicles or auditory placodes (AP) appear but they are void of otoliths.

Stage 22: formation of lens (Fig. 46)

A lens (L) appears in the optic cup.

Stage 23: appearance of otoliths (Fig. 47)

Otoliths (Ot) first appear in the auditory placodes.

Pharyngula period: marked by pigmentation of the eye, circulation, fin development.

Stage 24: pectoral fin, 26–27 somites (Fig. 48)

Body musculature continues to develop; a pectoral fin (PF) is evident.

Stage 25: pigmentation of retina (Fig. 49)

The primitive eye now possesses several layers of cells

within the retina during which the pigment layer (PL) of the retina first appears.

Stage 26: heart pumping (Fig. 50)

The heart (He) begins to pulsate, suggesting that it possesses well defined chambers. Blood carrying channels are sophisticated, but not mature.

Stage 27: lens becomes transparent (Fig. 51)

Originally cloudy or opaque, the lens (L) of each eye is now transparent.

Stage 28: openings of mouth and anus (Fig. 52)

A thin epithelium originally covered the mouth and anus, but by this time, the mouth opening (M) and the anus are now open, indicating that the gastro-intestinal tract is either complete or near completion.

Stage 29: peritoneal wall pigmentation (Fig. 53)

The peritoneum, which lines the body cavity, begins to acquire pigment granules (PG) or pigment cells (melanophores). Anterior pharyngeal arches (PA) forming lower jaw containing Meckel's cartilage (Mec) are evident. By this time, the liver and other body cavity organs may be discernible within the body cavity.

Stage 30: depletion of yolk (Fig. 54)

Prior to hatching, depletion of the pink staining eosinophilic yolk (Y) occurs and is greatly reduced in size.

Hatching period: yolk depleted, hatching occurs just prior to or at the time of parturition.

Stage 31: prehatching (Fig. 55)

Pelvic fin (PF) is easily visible and the chorion membrane (CM) envelops the entire embryo.

Stage 32: hatching (Fig. 56)

Release of embryos from female.

Stage 33: newborn larva (Fig. 57)

Table 4

Comparison of rockfish development as noted by Yamada and Kusakari (1991) and Chaillé (2006). Periods of early development adopted from Kimmel et al. (1995). Yamada and Kusakari (1991) provide the basic developmental sequence. Brief embryonic development was described by Chaillé (2006) where he noted particular embryonic developmental differences with Yamada and Kusakari (1991). Yamada and Kusakari data presented with permission.

Developmental periods (Kimmel et al., 1995)		Developmental stages (Yamada and Kusakari, 1991)	(Chaillé, 2006)
	1	Mature unfertilized ovum	
Zygote period			
	2	Formation of the germ disc (blastodisc)	
Cleavage period			
	3	2-celled (embryo)	
	4	4-celled (embryo)	
	5	8-celled (embryo)	
	6	16-celled (embryo)	
	7	32-celled (embryo)	
	8	64-celled (embryo)	
Blastula period			
	9	Morula	
	10	Early blastula	
	11	Late blastula	
	12	Beginning of epiboly	
Gastrula period			
	13	Early gastrula	
	14	Late gastrula	
Segmentation period			
	15	Embryonic shield	
	16	Head fold	
	17	Optic vessicles	
	18	Beginning of somite formation	optic vesicles, 10 somites
	19	Finfold	
	20	Optic cups, 22–23 somites	
	21	Auditory placodes	
	22	Formation of lens, motility	lens, auditory placodes
	23	Appearance of otoliths	
Pharyngula period			
	24	Pectoral fins, 26–27 somites	otoliths, 25–27 somites
	25	Pigmentation of retina	
	26	Blood circulation (heart pumping)	
	27	Lens becomes transparent	transparent lens, blood circulation
	28	Openings of the mouth and anus	
	29	Pigmentation of the peritoneal wall	
	30	Depletion of yolk, 5.4 mm body length	fully formed urinary tract, reduction of yolk begins
Hatching period			
	31	Prehatching	full somites, teeth visible; pre-hatching peristalsis
	32	Hatching	
	33	Newborn larva, 6.2 mm body length	

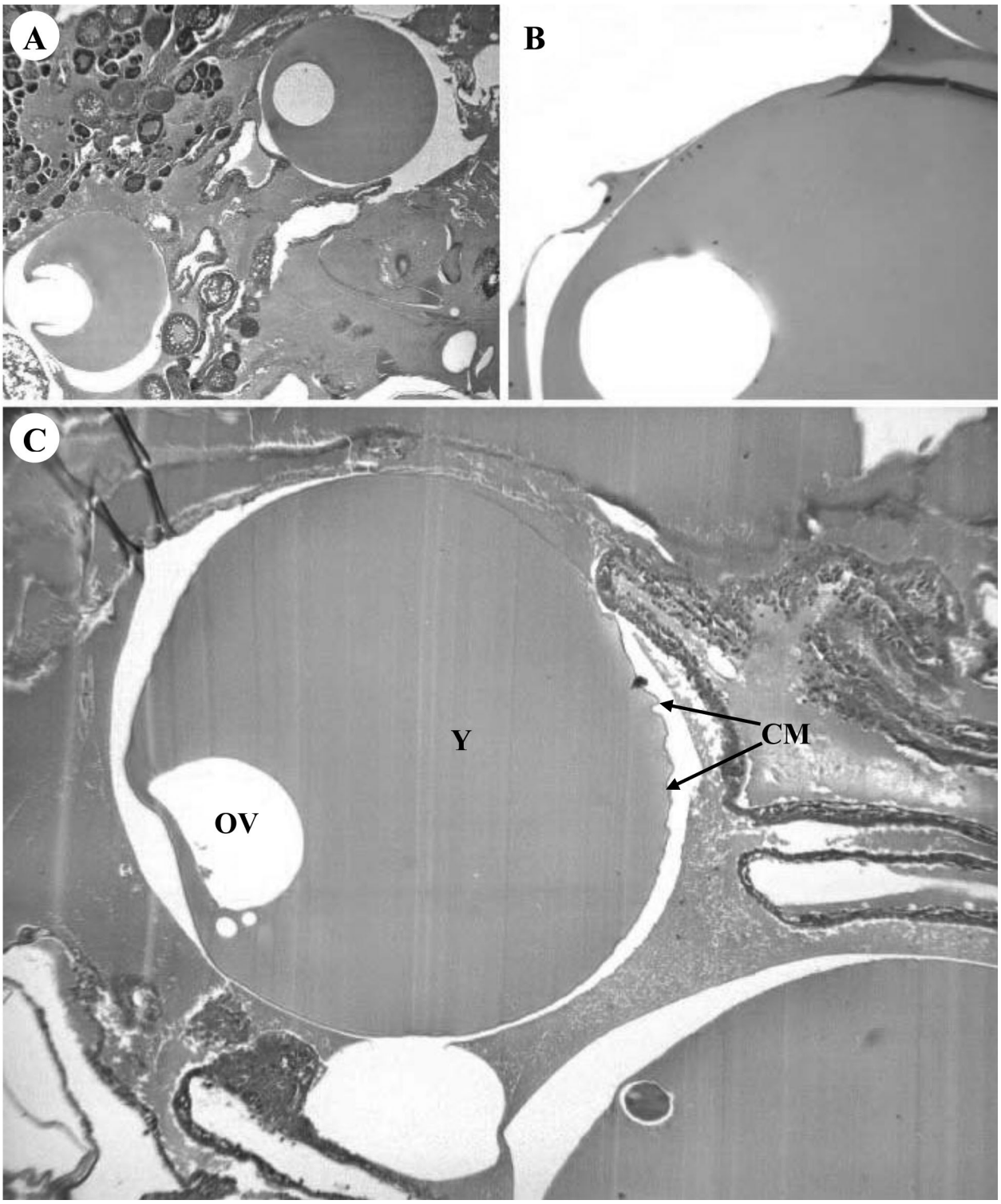

Figure 25

Mature ovum (embryonic development stage 1). *Sebastes alutus* collected 19 March 2004, 36 cm, 680 g. (A) 25×, (B) 160×, and (C) 100×.

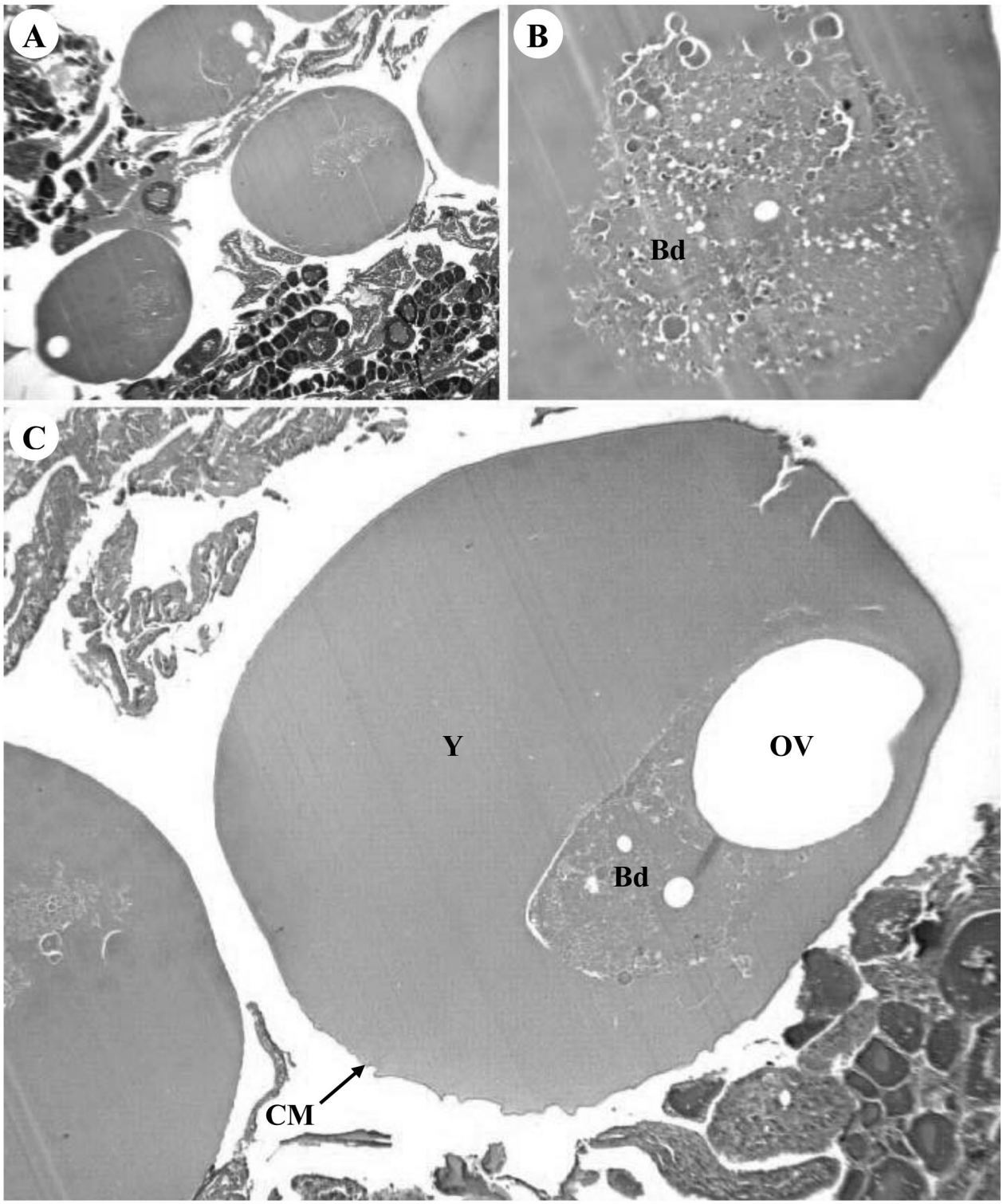

Figure 26

Formation of blastodisc (embryonic development stage 2). *Sebastes alutus* collected 19 March 2004, 32 cm, 460 g. (A) 25×, (B) 200×, and (C) 100×.

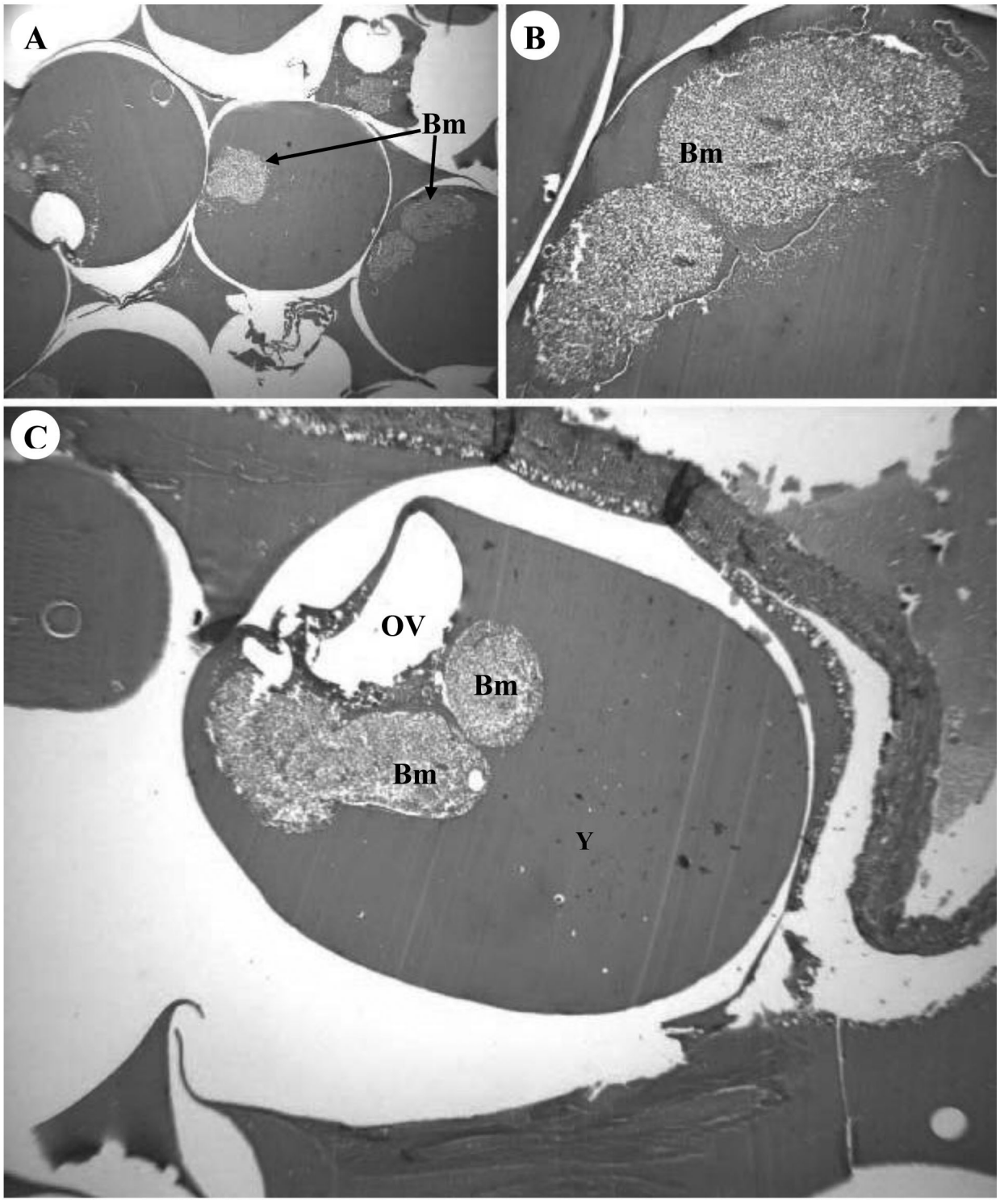

Figure 27

2-celled embryo (embryonic development stage 3). *Sebastes alutus* collected 18 March 2004, 40 cm, 940 g. (A) 25×, (B) 200×, and (C) 100×.

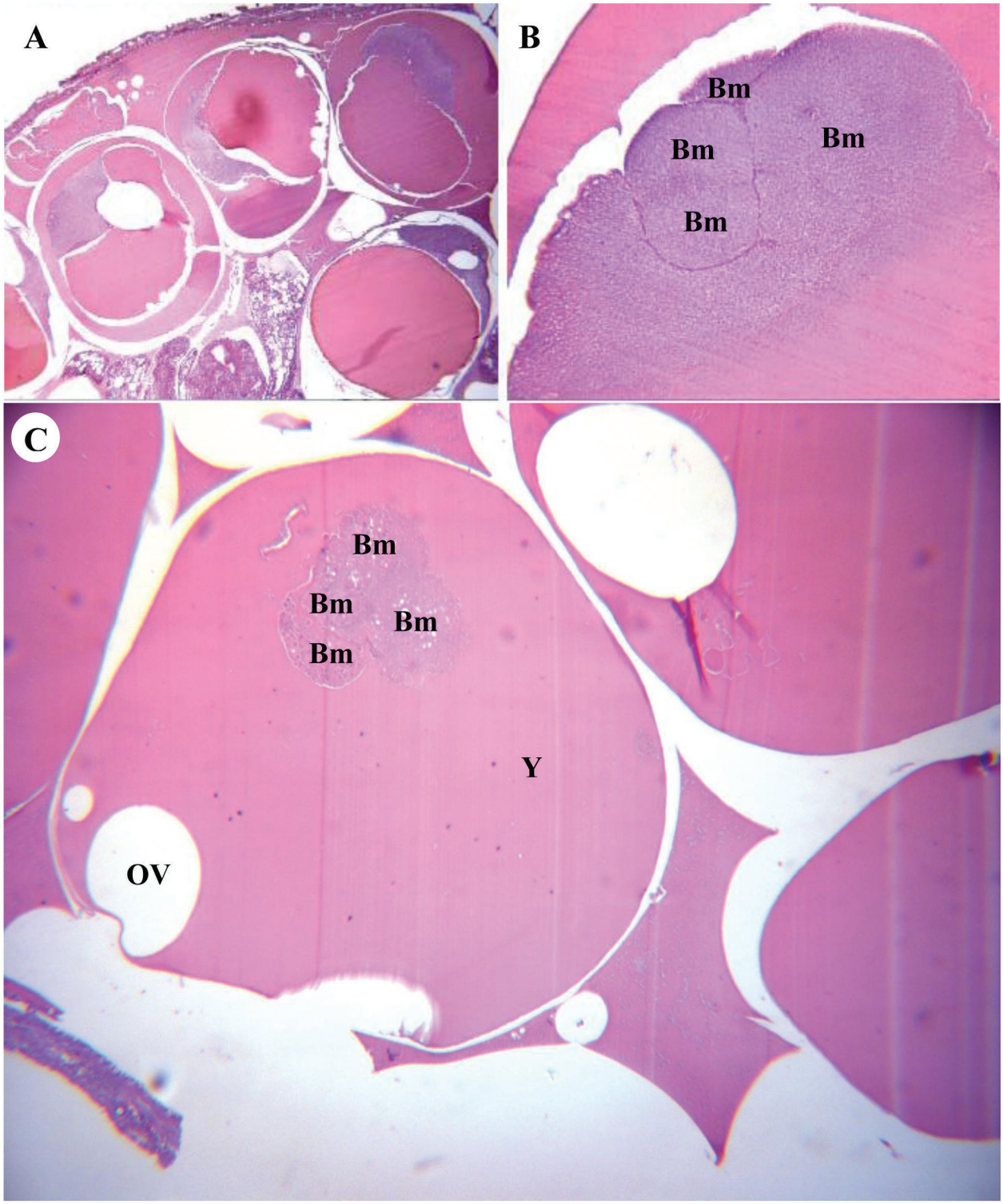

Figure 28

4-celled embryo (embryonic development stage 4). *Sebastes alutus* collected 25 March 2004, 38 cm, 840 g. (A) 25×, (B) 200×, and (C) 100×.

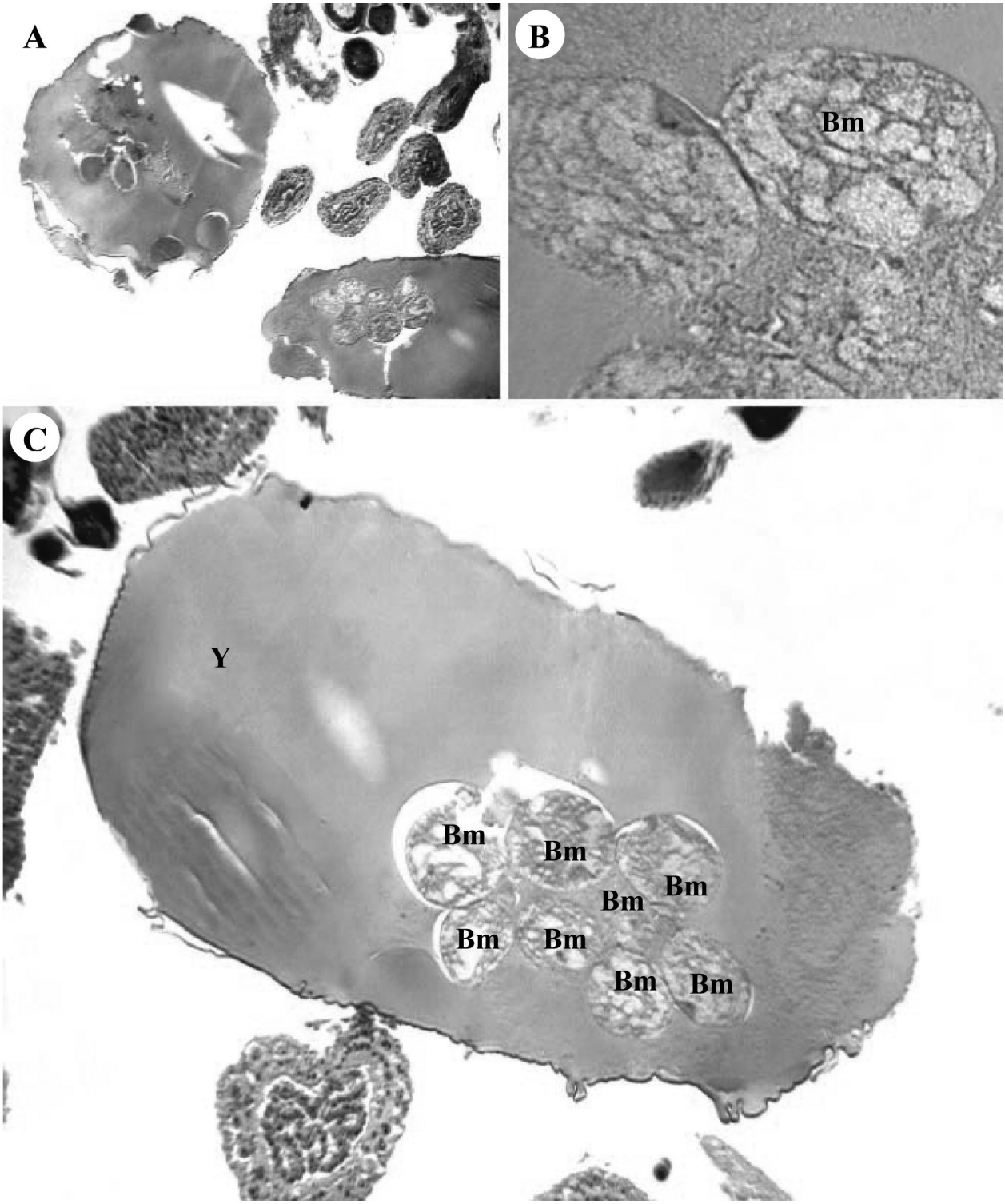

Figure 29

8-celled embryo (embryonic development stage 5). *Sebastes alutus* collected 27 March 2004, 38 cm, 700 g. (A) 25×, (B) 200×, and (C) 100×.

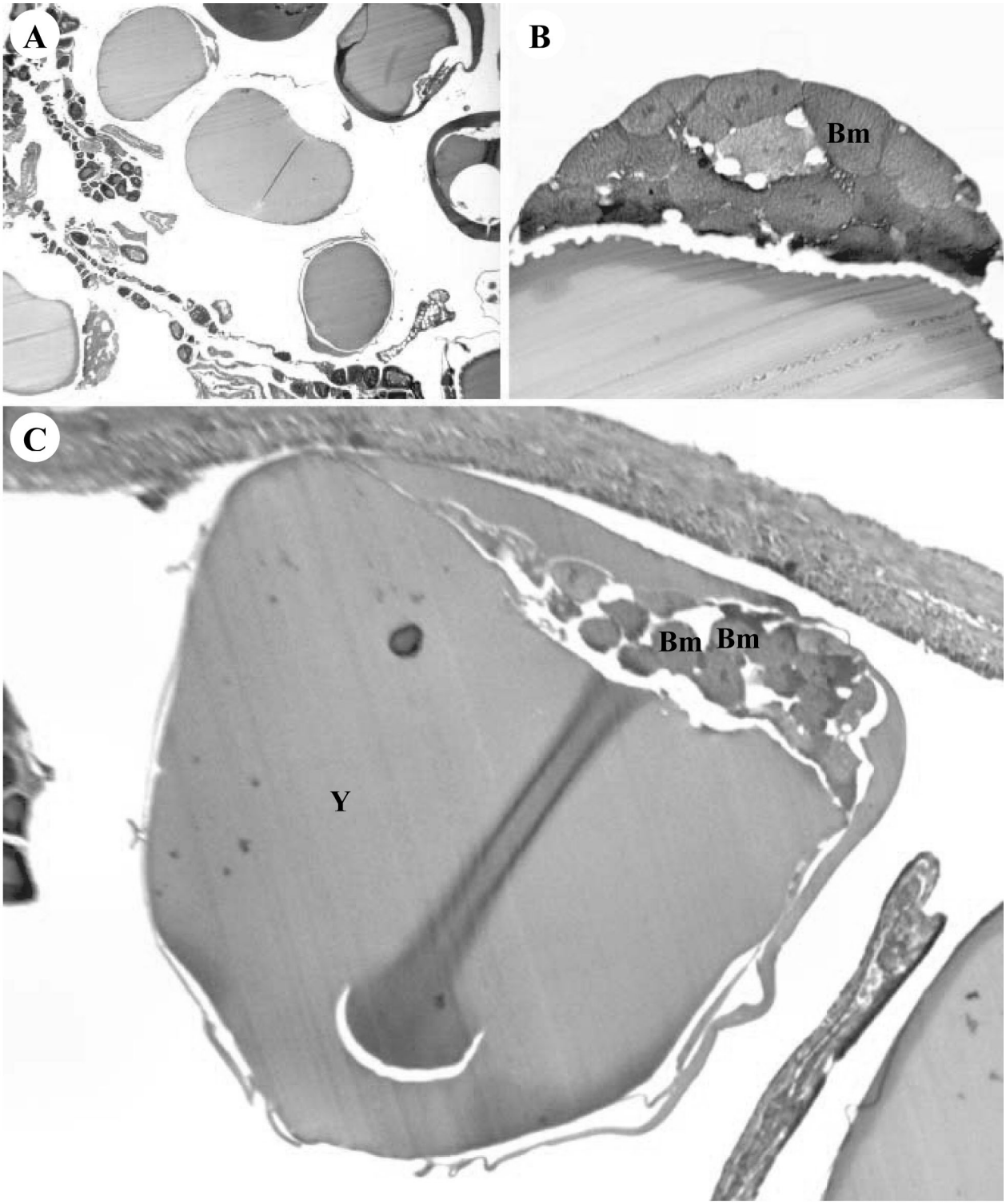

Figure 30

16-celled embryo (embryonic development stage 6). *Sebastes alutus* collected 17 March 2004, 37 cm, 660 g. (A) 25×, (B) 200×, and (C) 100×.

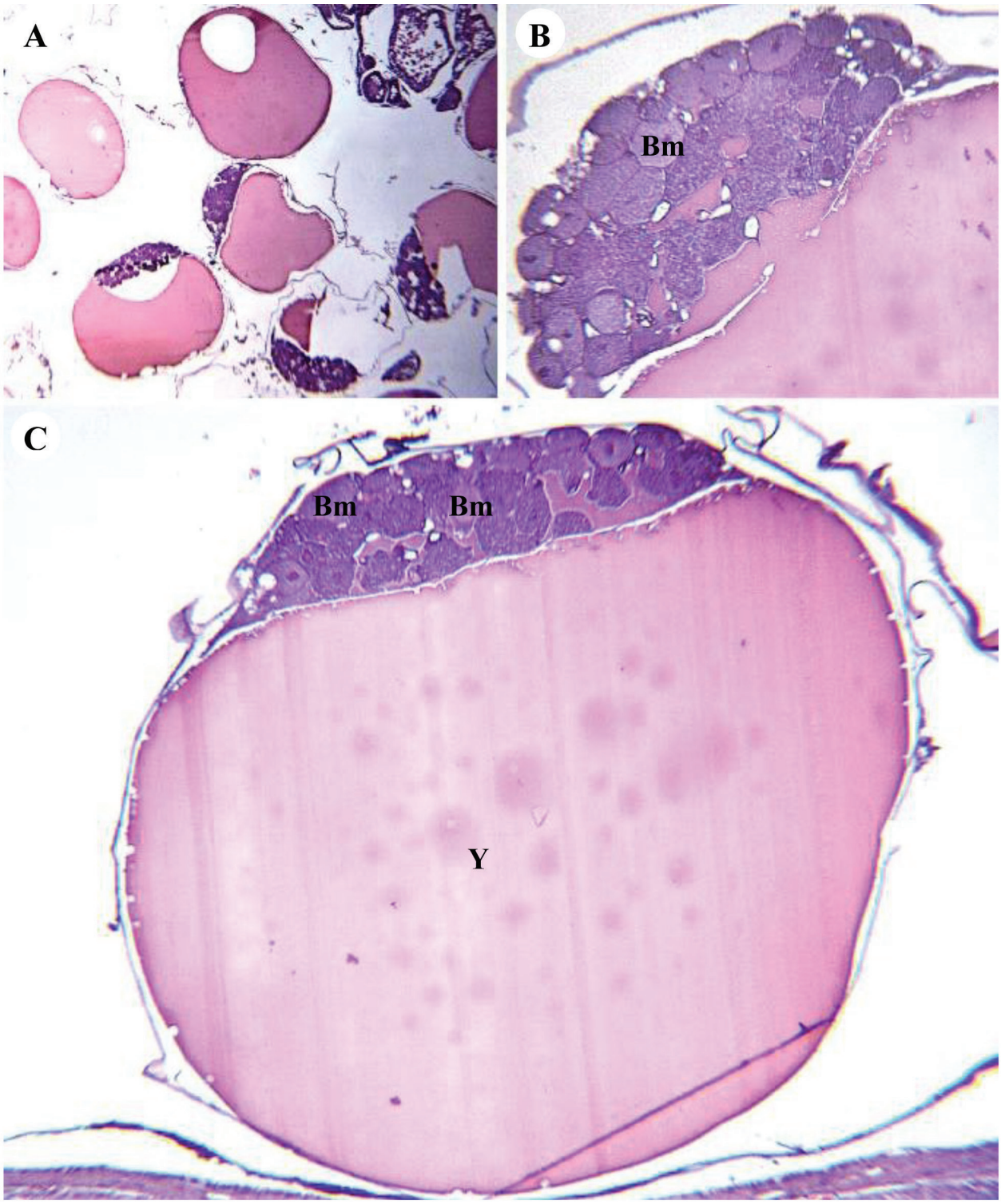

Figure 31

32-celled embryo (embryonic development stage 7). *Sebastes alutus* collected 25 March 2004, 33 cm, 520 g. (A) 25×, (B) 160×, and (C) 100×.

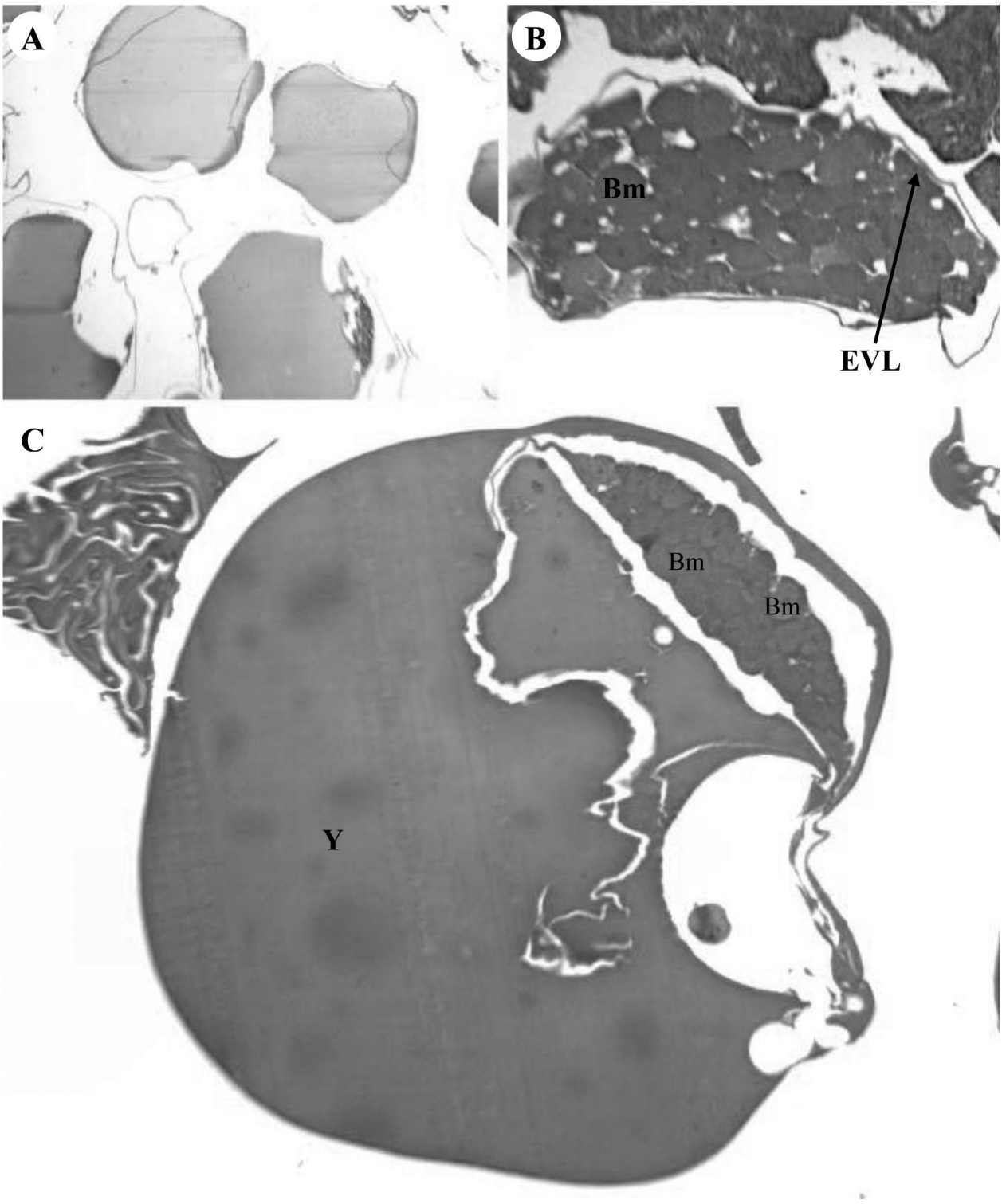

Figure 32

64-celled embryo (embryonic development stage 8). *Sebastes alutus* collected 19 March 2004, 33 cm, 540 g. (A) 25×, (B) 160×, and (C) 100×.

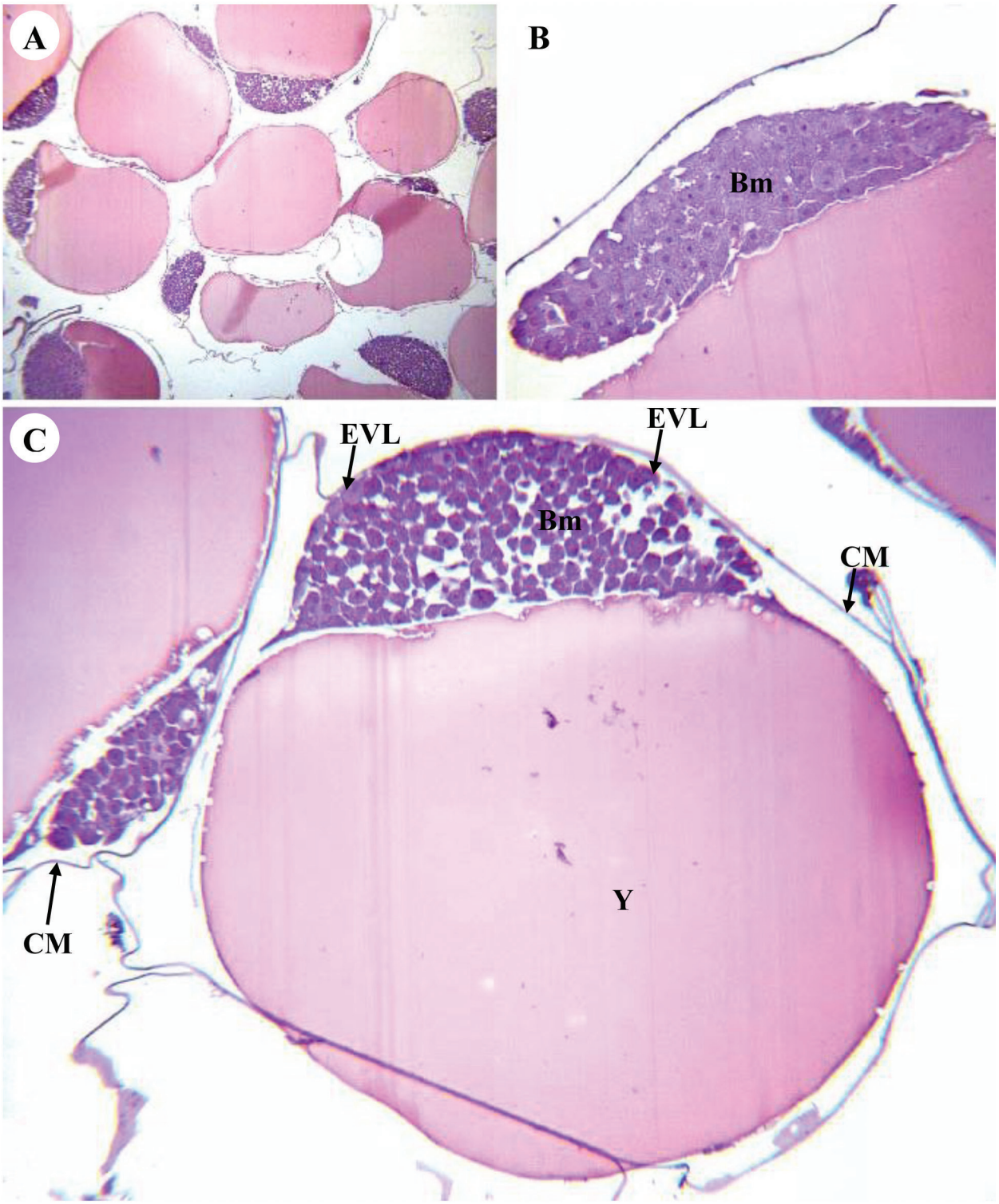

Figure 33

Morula (embryonic development stage 9). *Sebastes alutus* collected 22 March 2004, 34 cm, 520 g. (A) 25×, (B) 160×, and (C) 100×.

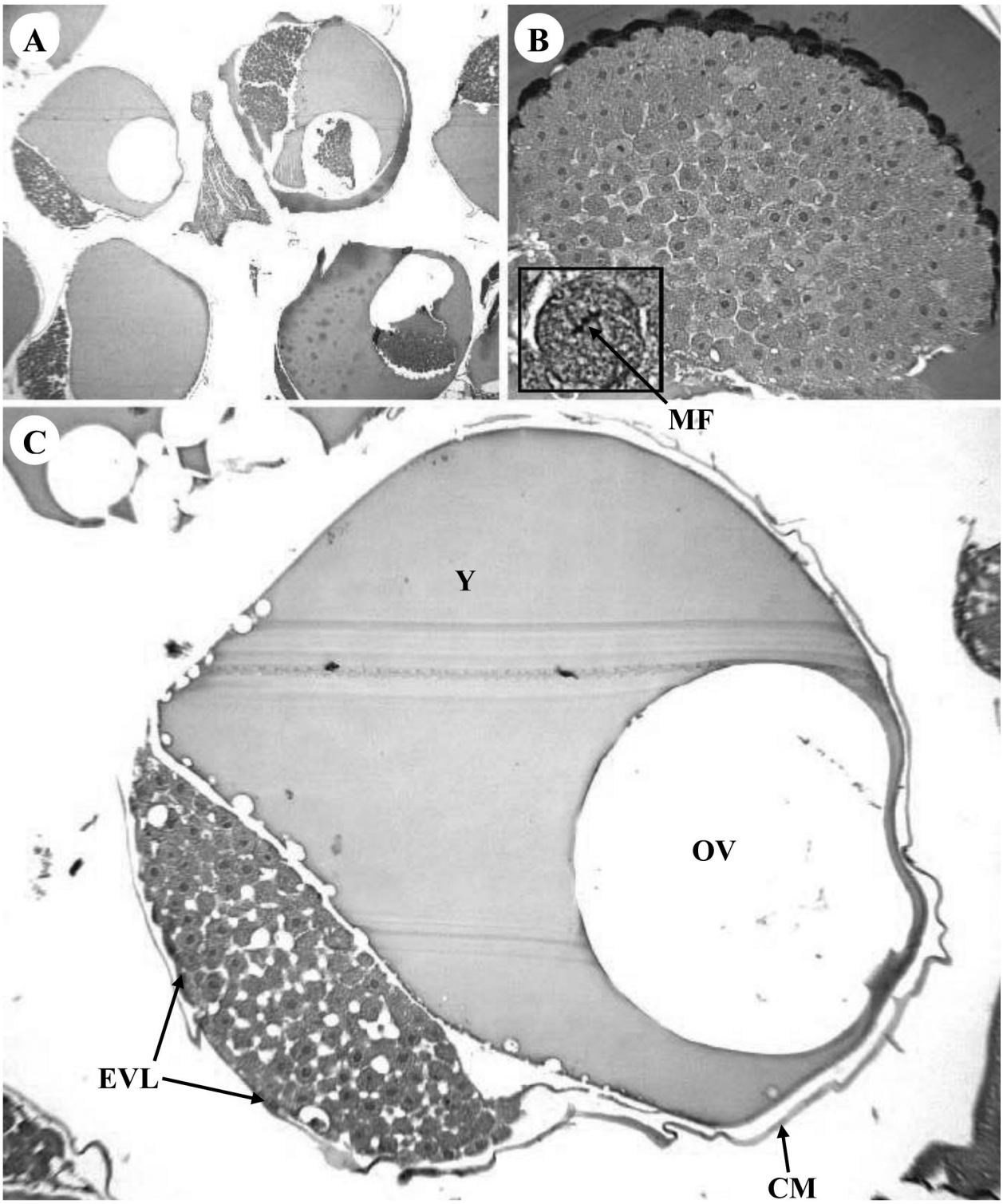

Figure 34

Early blastula (embryonic development stage 10). *Sebastes alutus* collected 22 March 2004, 34 cm, 620 g. (A) 25×, (B) 100×, inset illustrates mitotic figures, and (C) 100×.

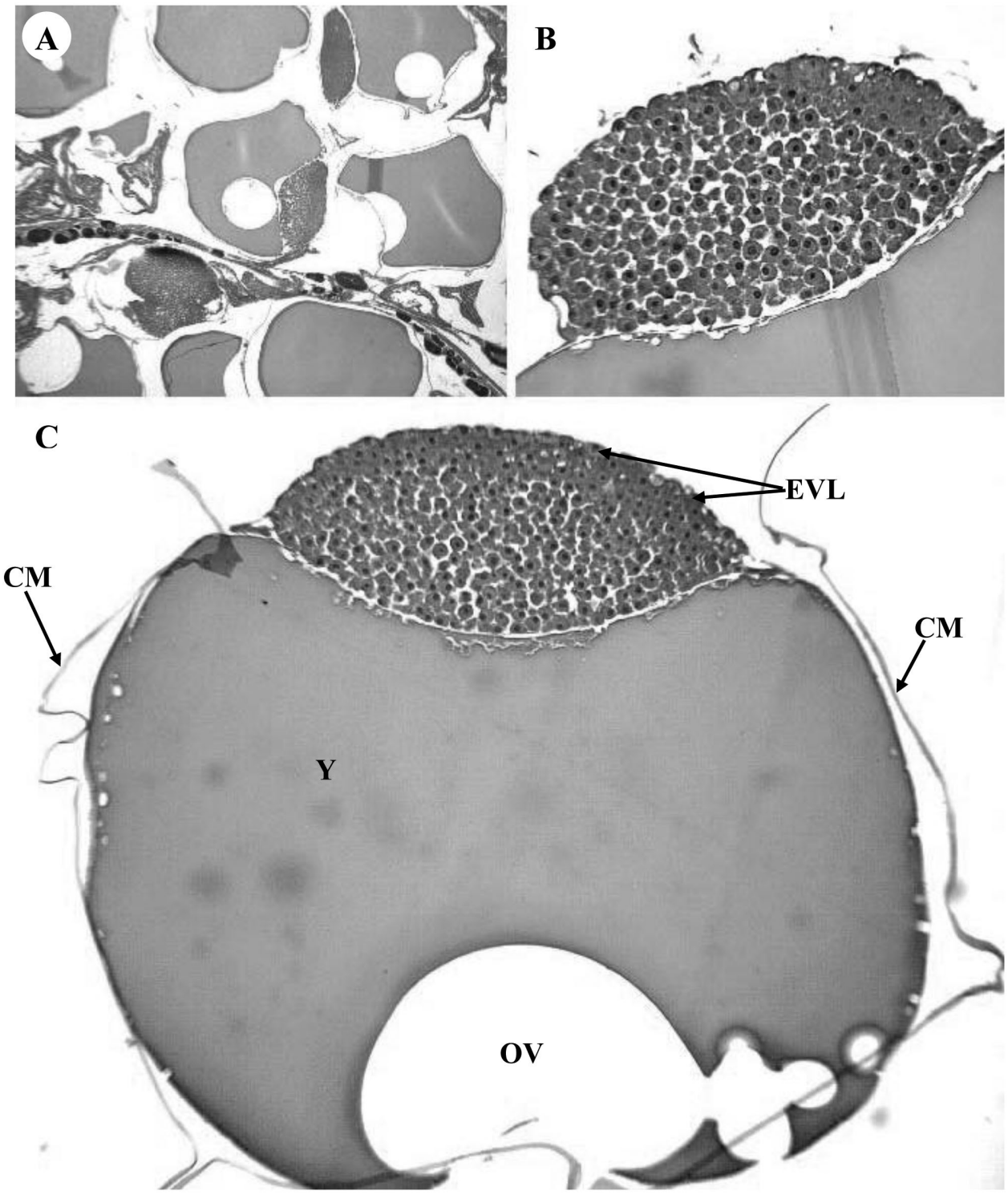

Figure 35

Late blastula (embryonic development stage 11). *Sebastes alutus* collected 25 January 2004, 39 cm, 740 g. (A) 25×, (B) 100×, and (C) 100×.

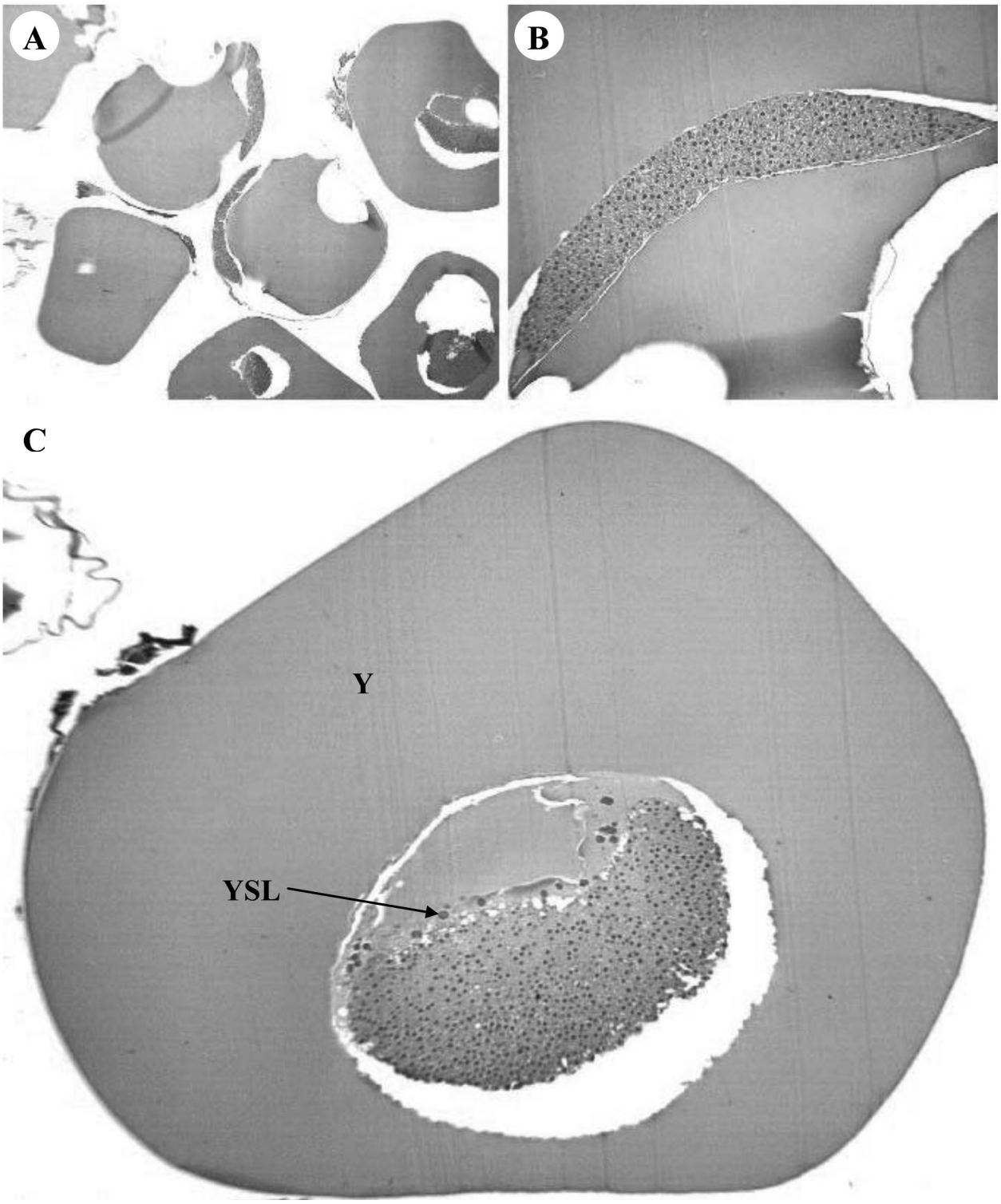

Figure 36

Beginning of epiboly (embryonic development stage 12). *Sebastes alutus* collected 26 January 2004, 36 cm, 580 g. (A) 25×, (B) 100×, and (C) 100×.

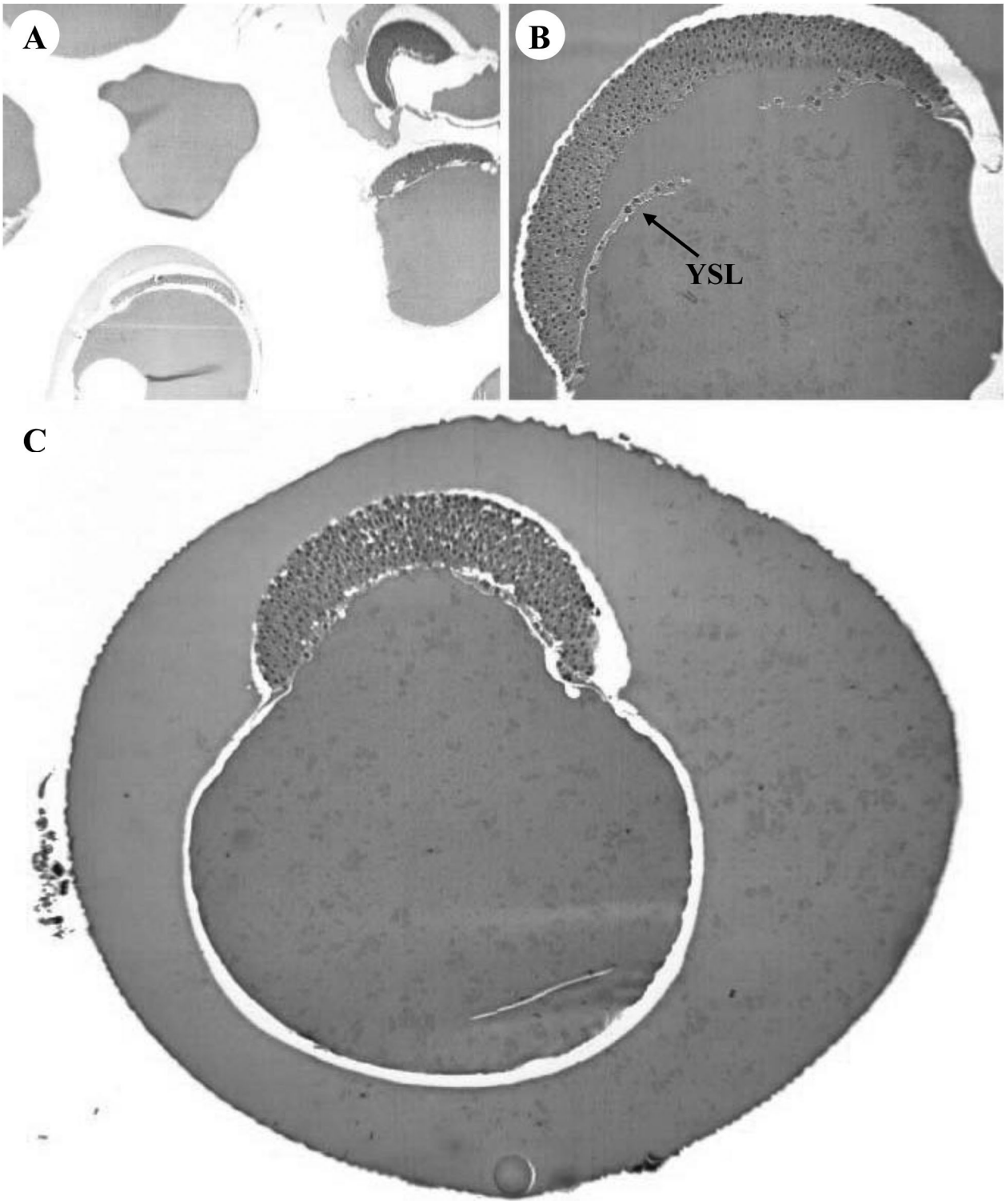

Figure 37

Early gastrula (embryonic development stage 13). *Sebastes alutus* collected 26 January 2004, 38 cm, 640 g. (A) 25×, (B) 100×, and (C) 100×.

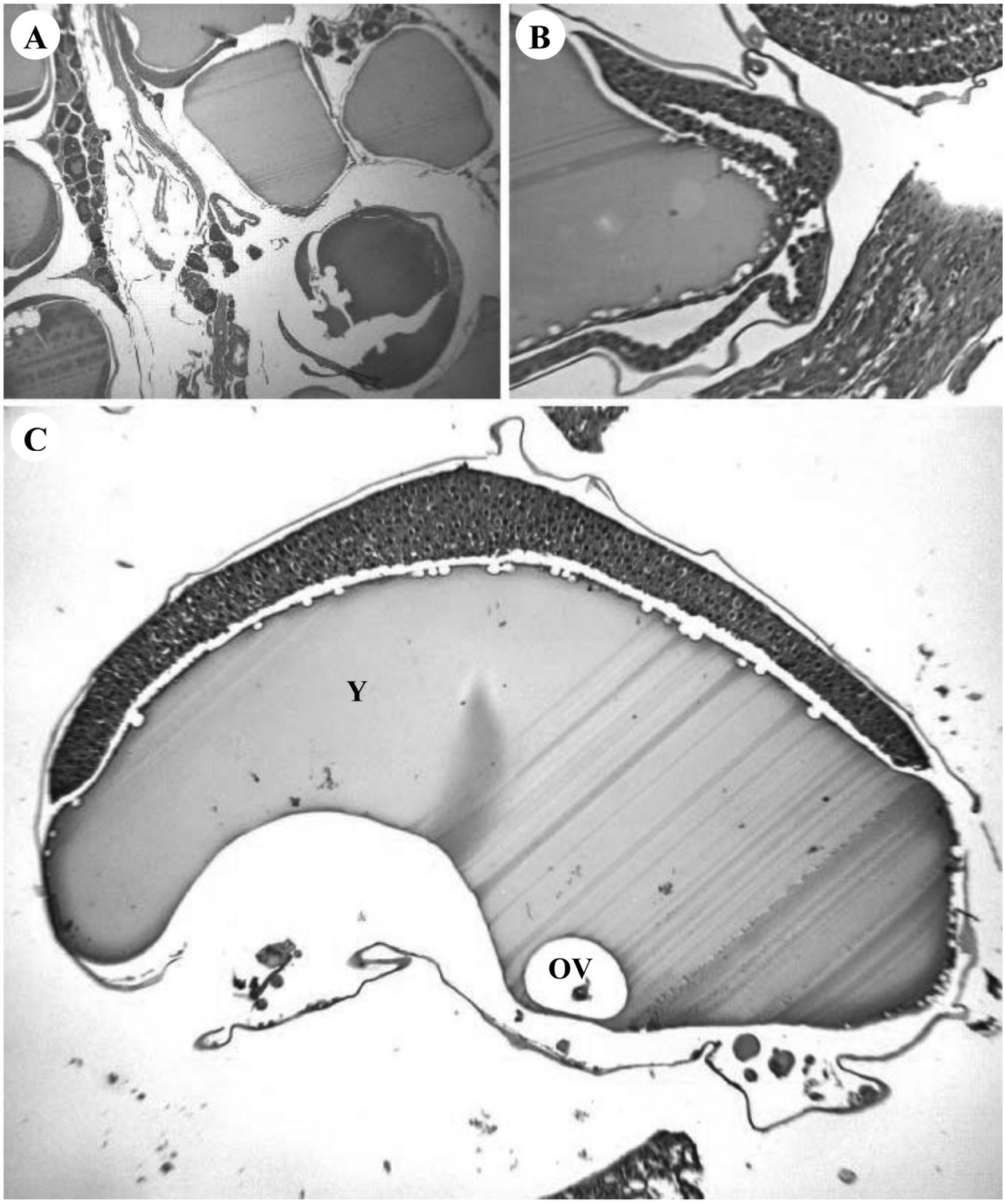

Figure 38

Late gastrula (embryonic development stage 14). *Sebastes alutus* collected 26 January 2004, 30 cm, 330 g. (A) 25×, (B) 100×, and (C) 100×.

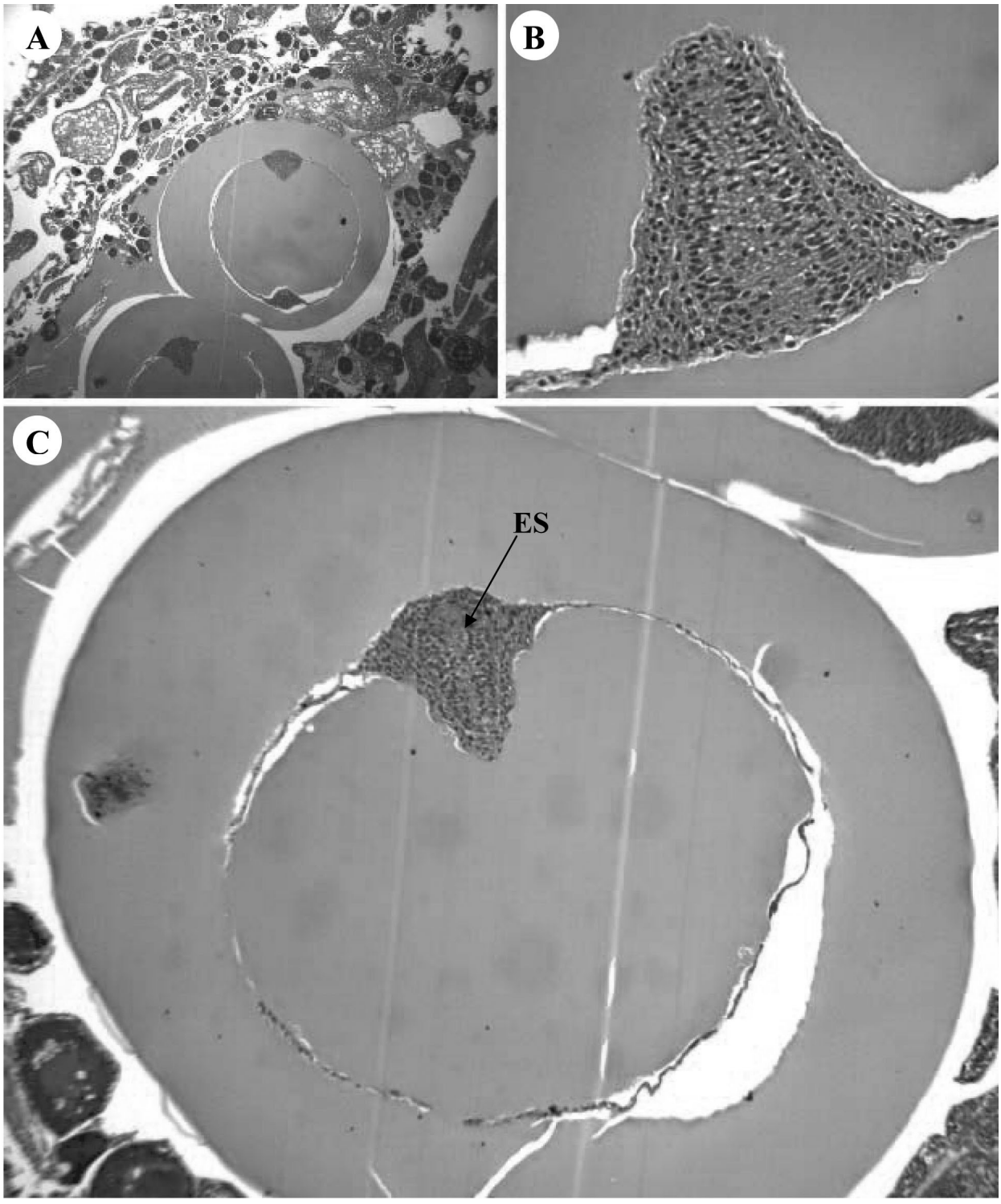

Figure 39

Embryonic shield (embryonic development stage 15). *Sebastes alutus* collected 23 January 2004, 34 cm, 520 g. (A) 25×, (B) 200×, and (C) 100×.

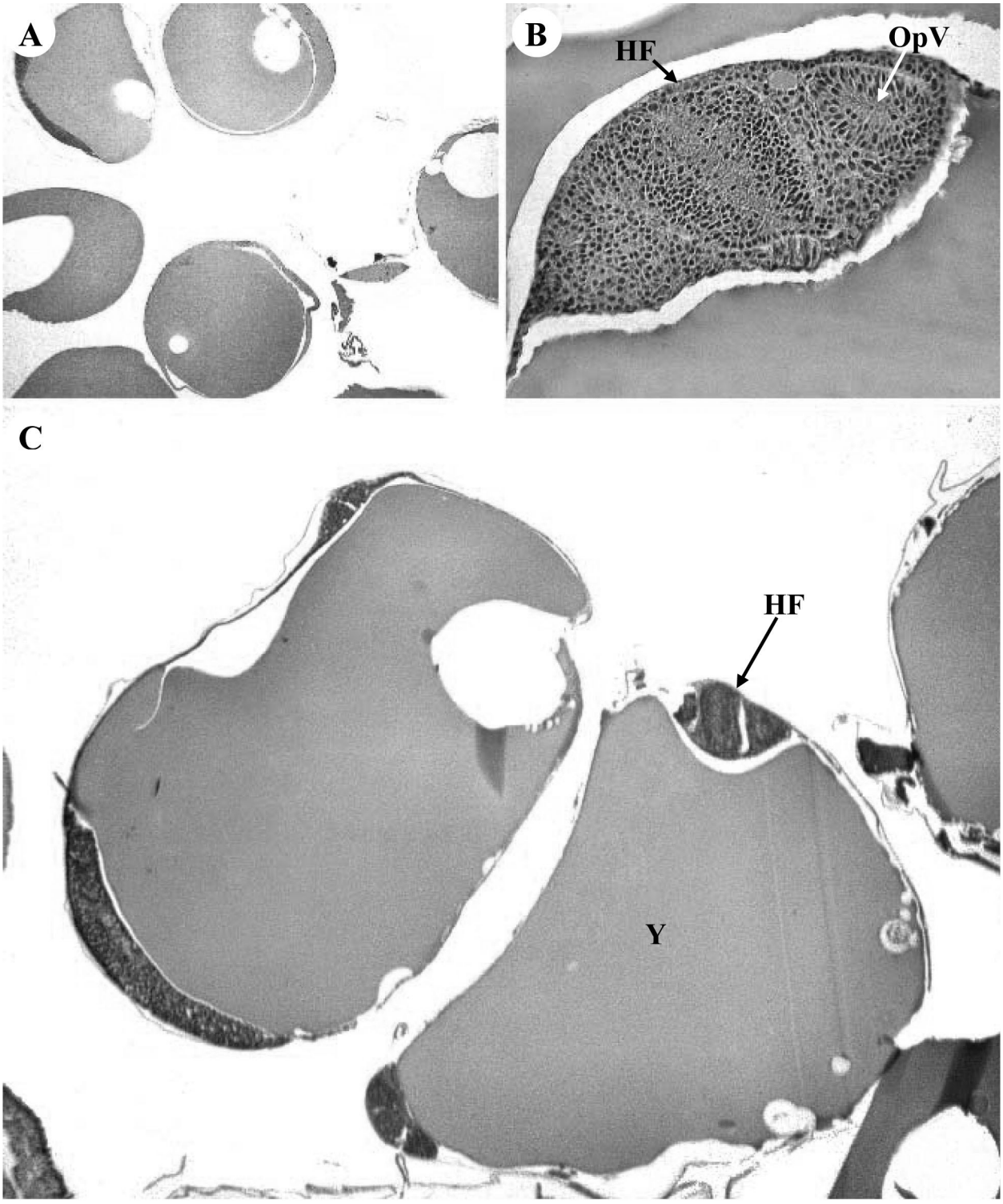

Figure 40

Head fold and developing optic vesicle (embryonic development stage 16 in transition to stage 17). *Sebastes alutus* collected 26 January 2004, 30 cm, 340 g. (A) 25×; (B) 200×; and (C) 100×.

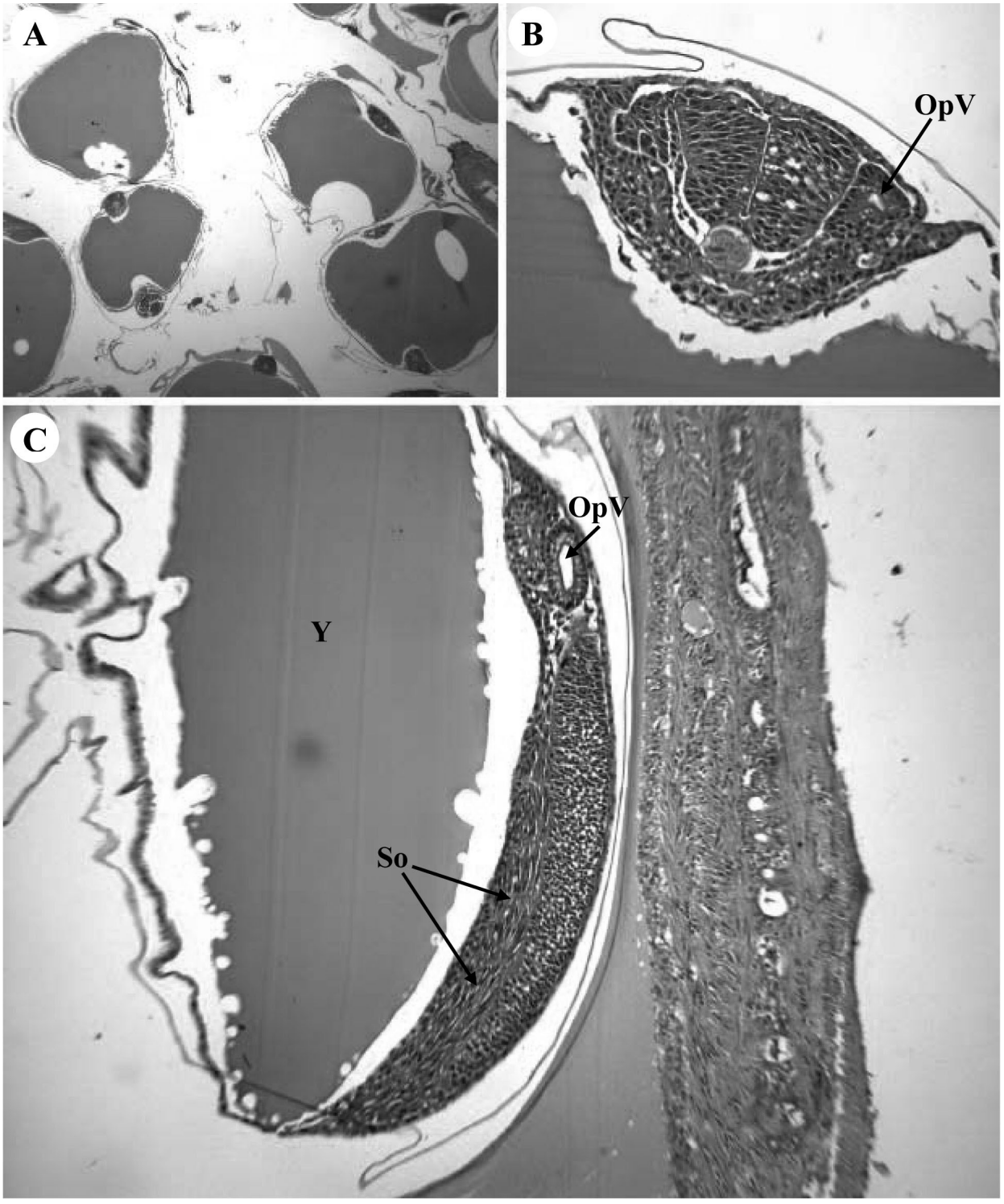

Figure 41

Optic vesicles and somite formation (embryonic development stage 17 in transition to stage 18). *Sebastes alutus* collected 26 January 2004, 32 cm, 380 g. (A) 25×, (B) 200×, and (C) 100×.

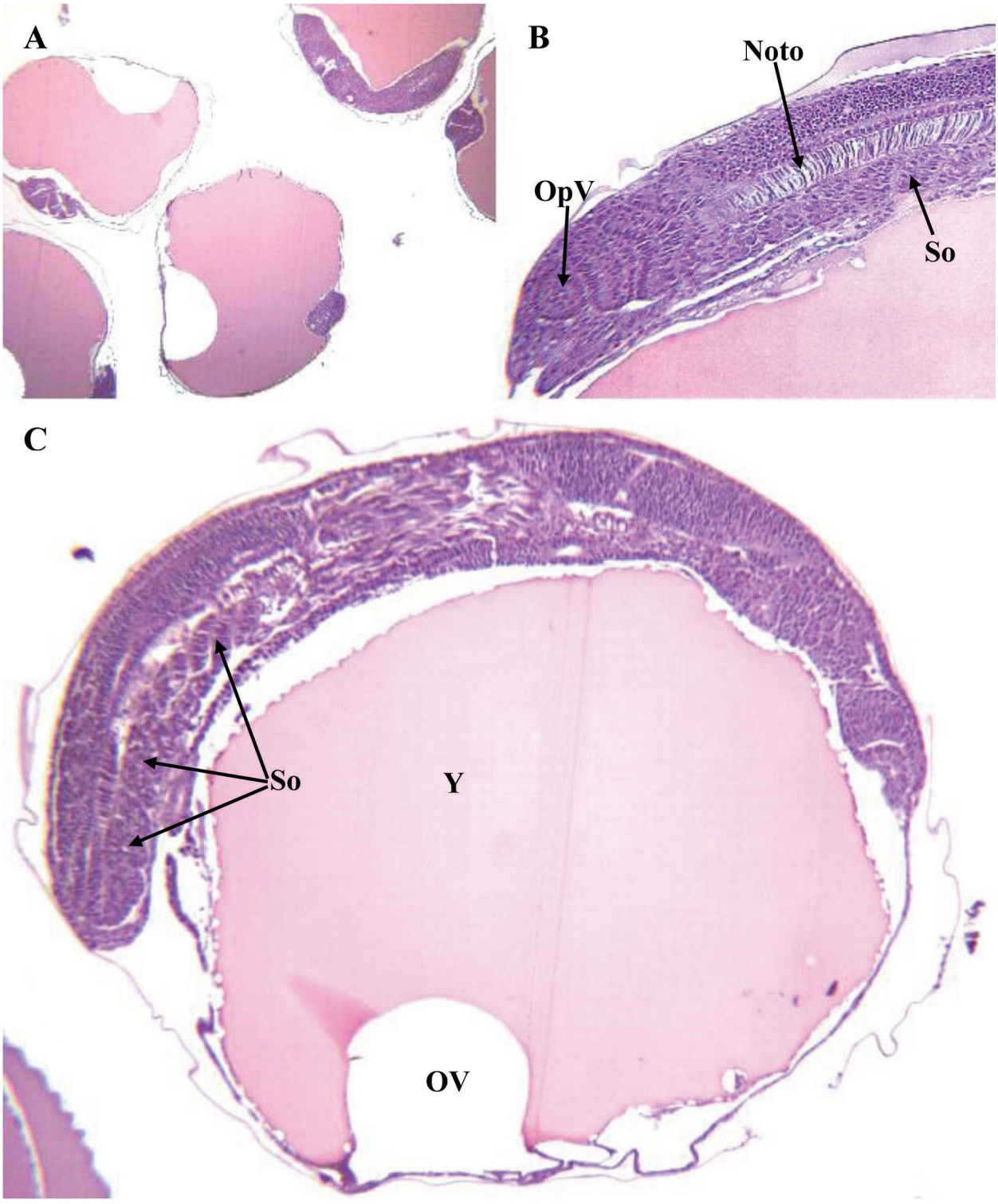

Figure 42

Somite formation begins (embryonic development stage 18). *Sebastes alutus* collected 26 January 2004, 36 cm, 560 g. (A) 25×, (B) 100×, and (C) 100×.

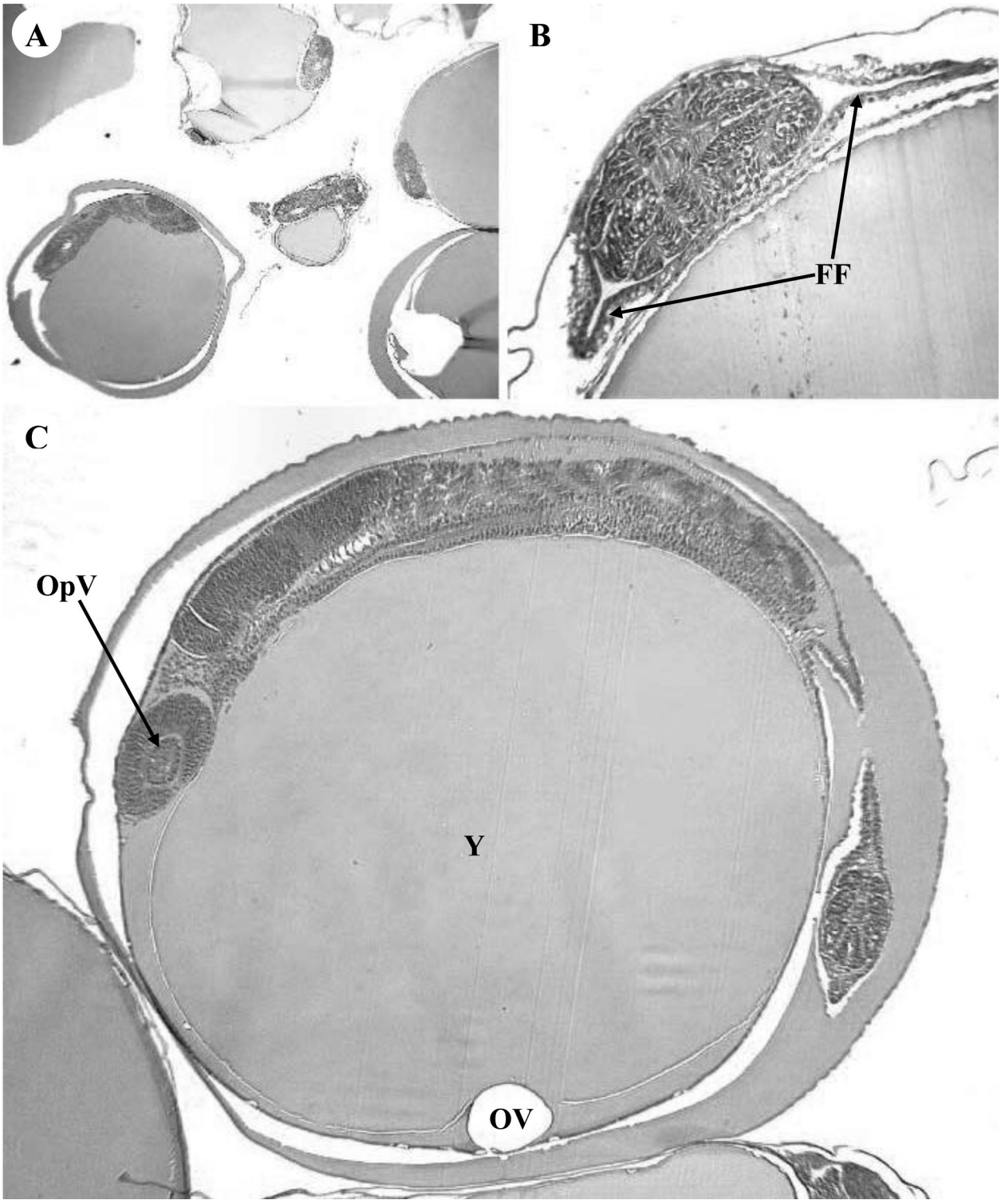

Figure 43

Fin fold (embryonic development stage 19). *Sebastes alutus* collected 26 January 2004, 37 cm, 600 g. (A) 25×, (B) 200×, and (C) 100×.

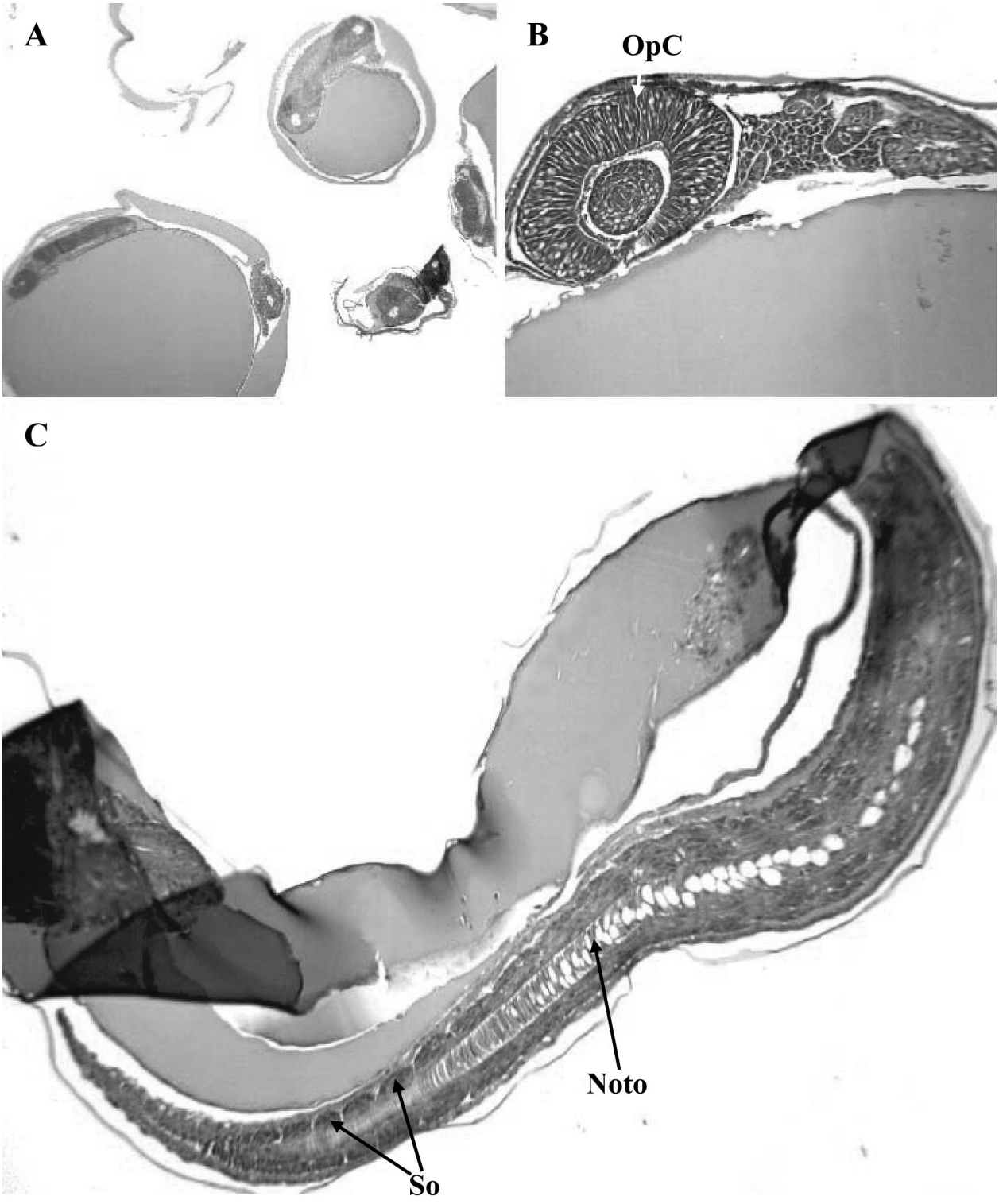

Figure 44

Optic cups, 22-23 somites (embryonic development stage 20). *Sebastes alutus* collected 26 January 2004, 35 cm, 540 g. (A) 25×, (B) 160×, and (C) 100×.

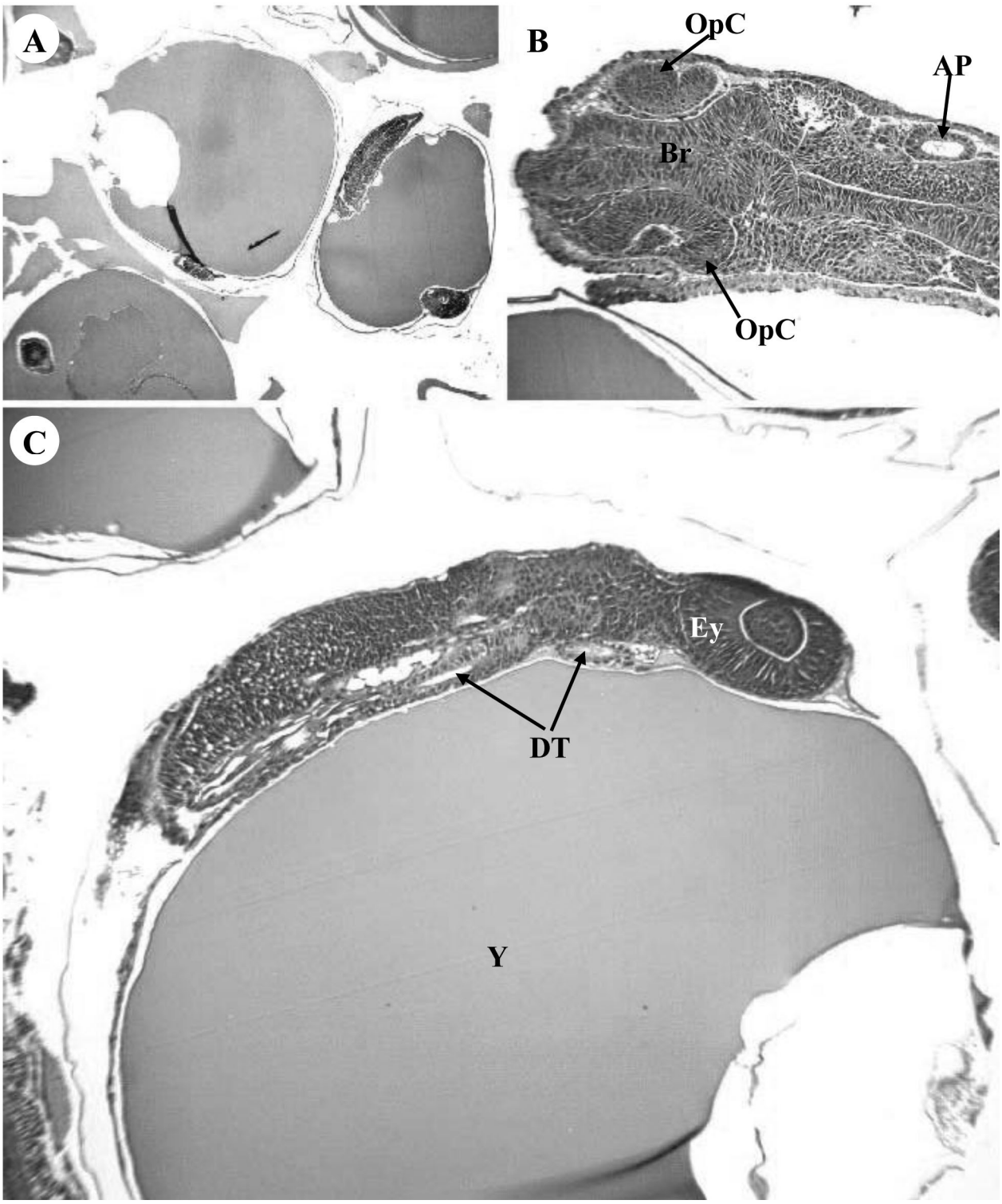

Figure 45

Auditory placodes (embryonic development stage 21). *Sebastes alutus* collected 26 January 2004, 36 cm, 560 g. (A) 25×, (B) 100×, and (C) 100×.

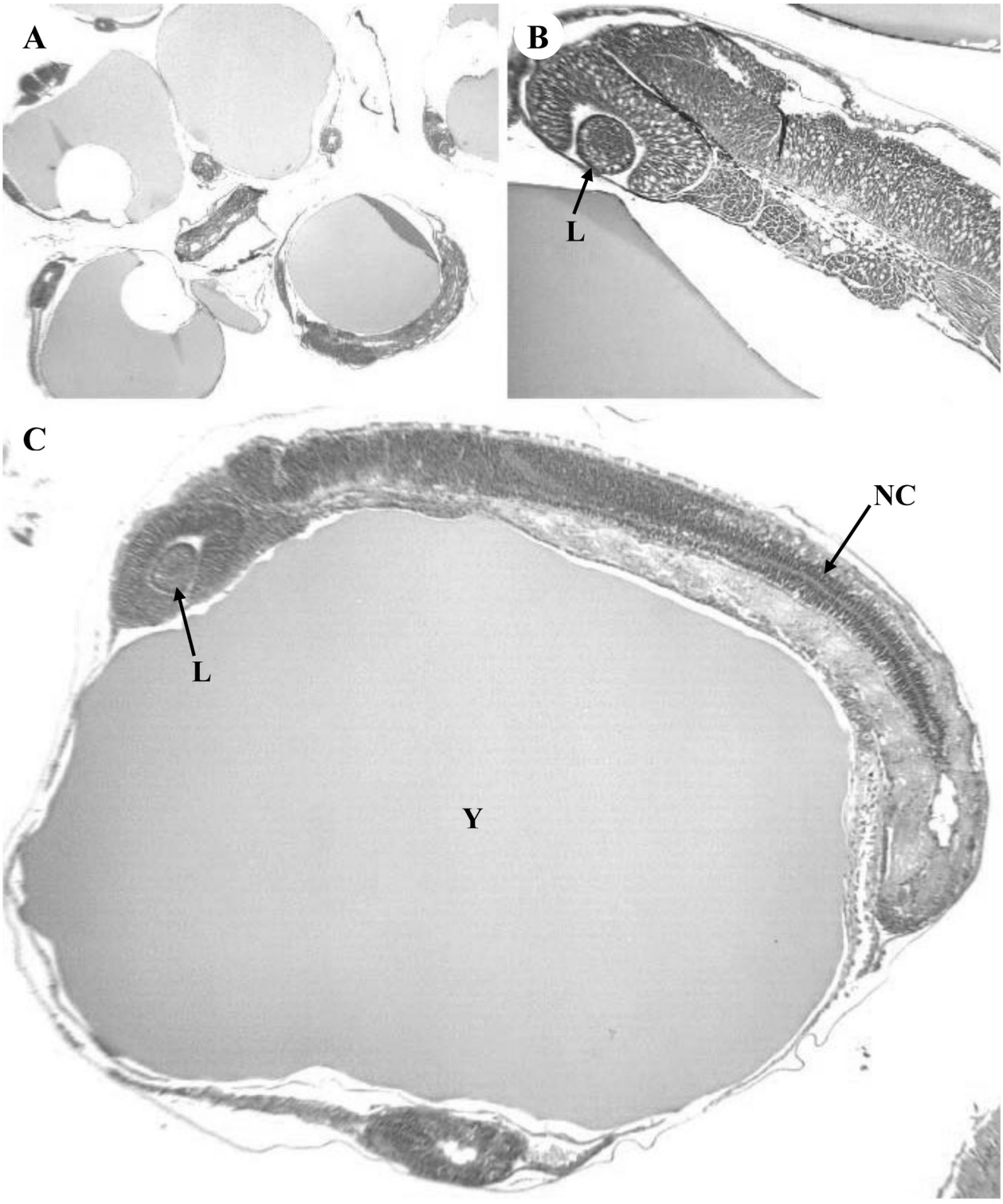

Figure 46

Formation of lens (embryonic development stage 22). *Sebastes alutus* collected 26 January 2004, 37 cm, 600 g. (A) 25×, (B) 100×, and (C) 100×.

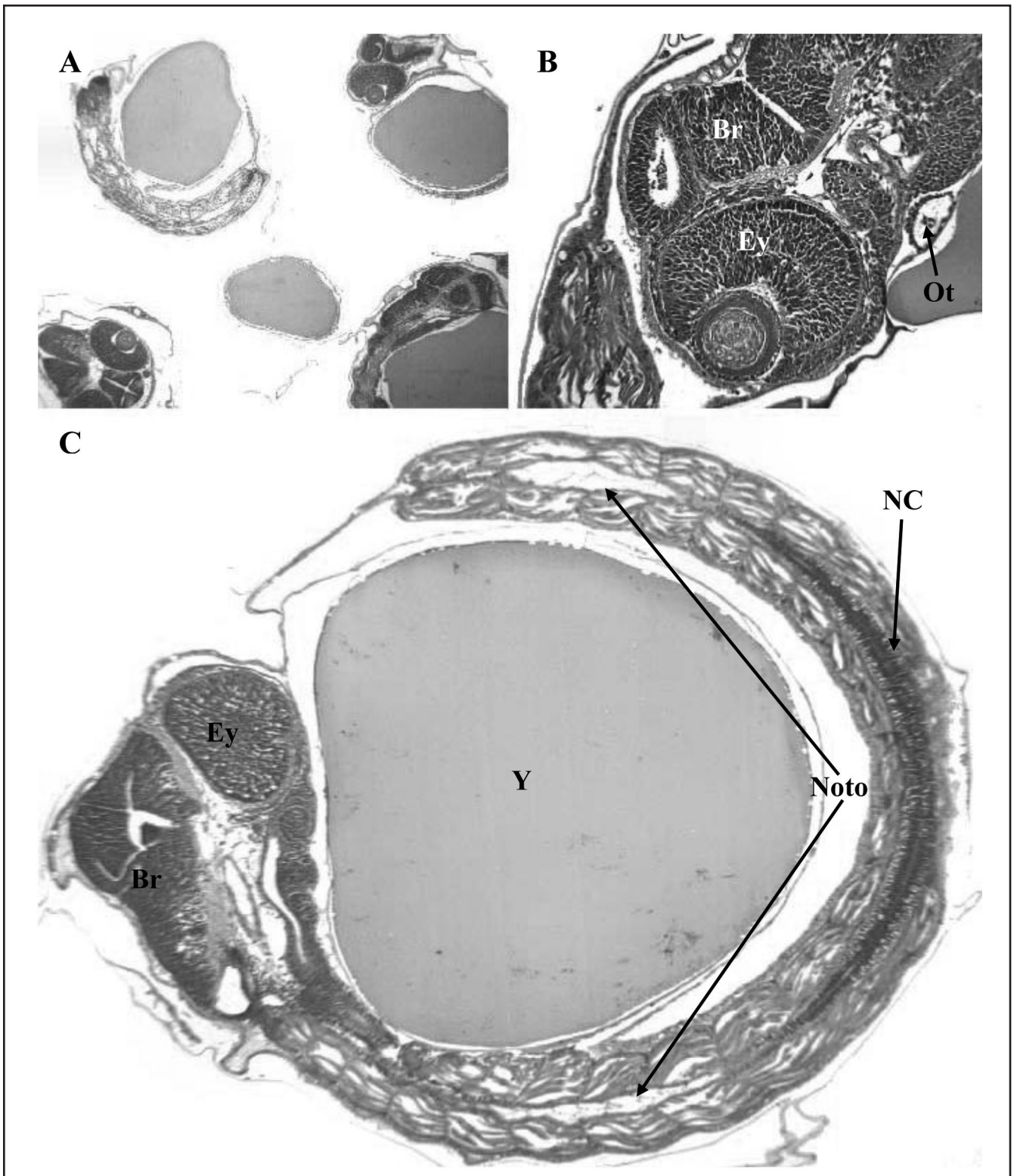

Figure 47

Appearance of otoliths (embryonic development stage 23). *Sebastes alutus* collected 16 March 2004, 39 cm, 880 g. (A) 25×, (B) 100×, and (C) 100×.

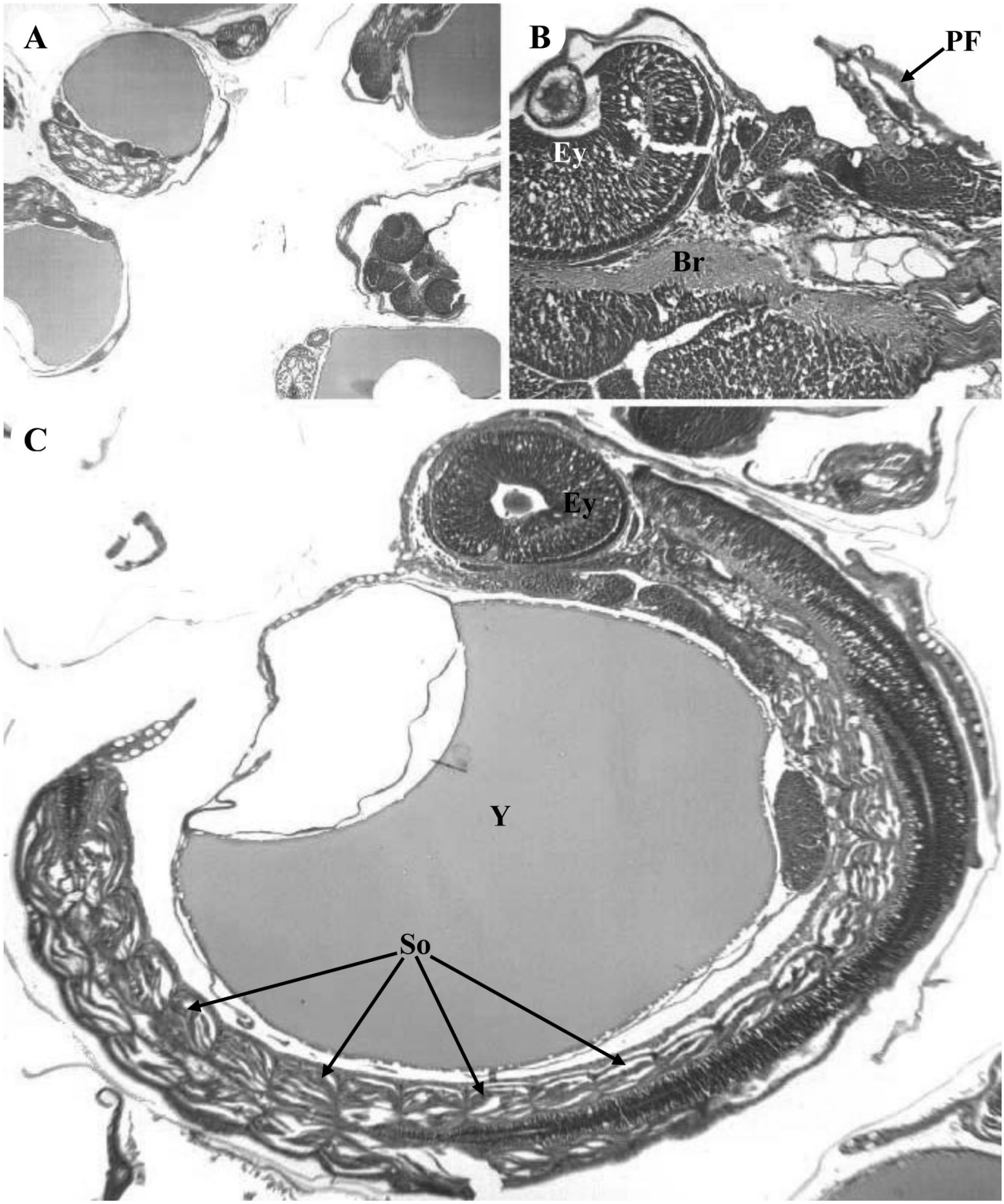

Figure 48

Pectoral fin, 26-27 somites (embryonic development stage 24). *Sebastes alutus* collected 19 March 2004, 41 cm, 1040 g. (A) 25×, (B) 160×, and (C) 100×.

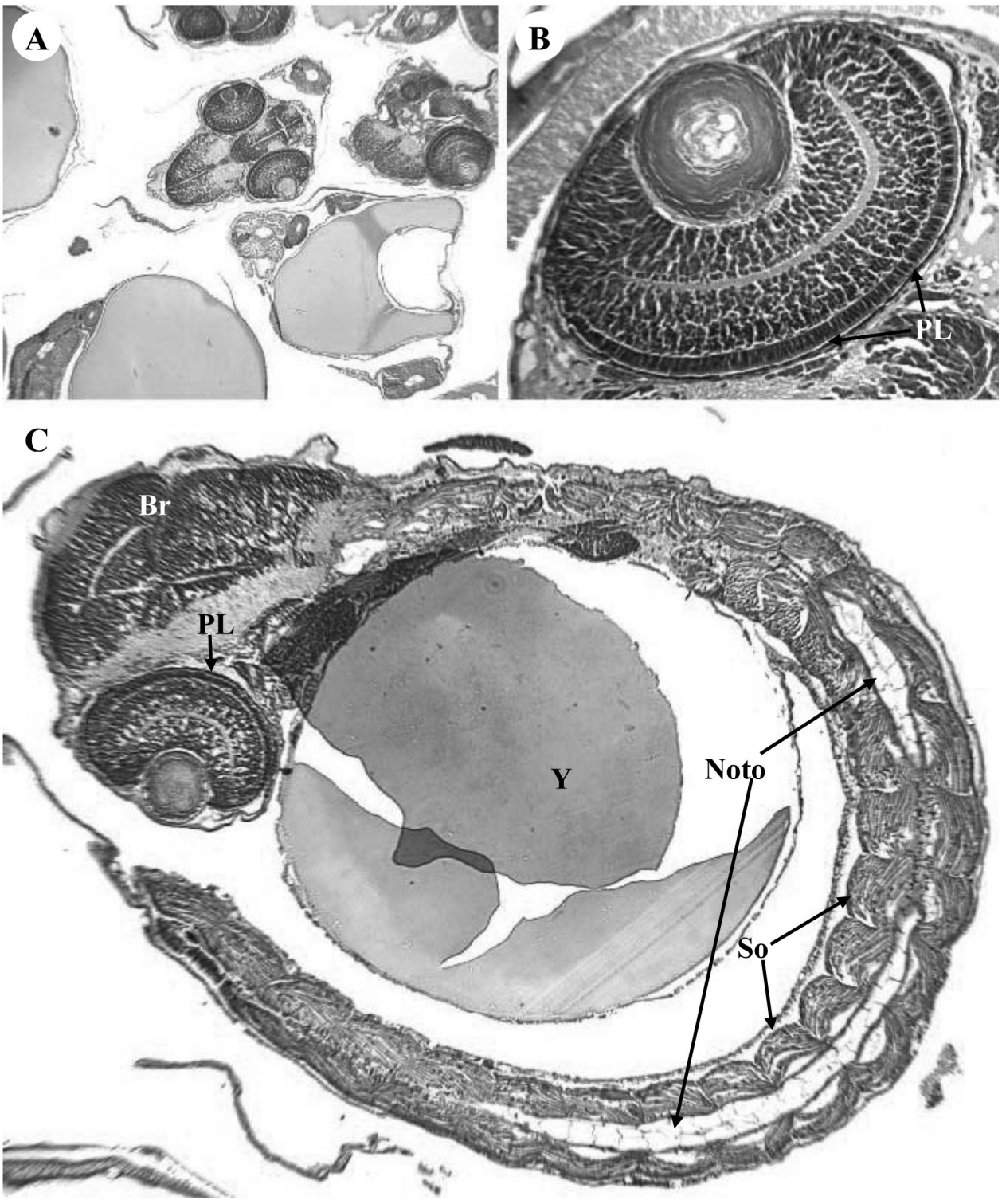

Figure 49

Pigmentation of retina (embryonic development stage 25). *Sebastes alutus* collected 25 March 2004, 38 cm, 840 g. (A) 25×, (B) 200×, and (C) 100×.

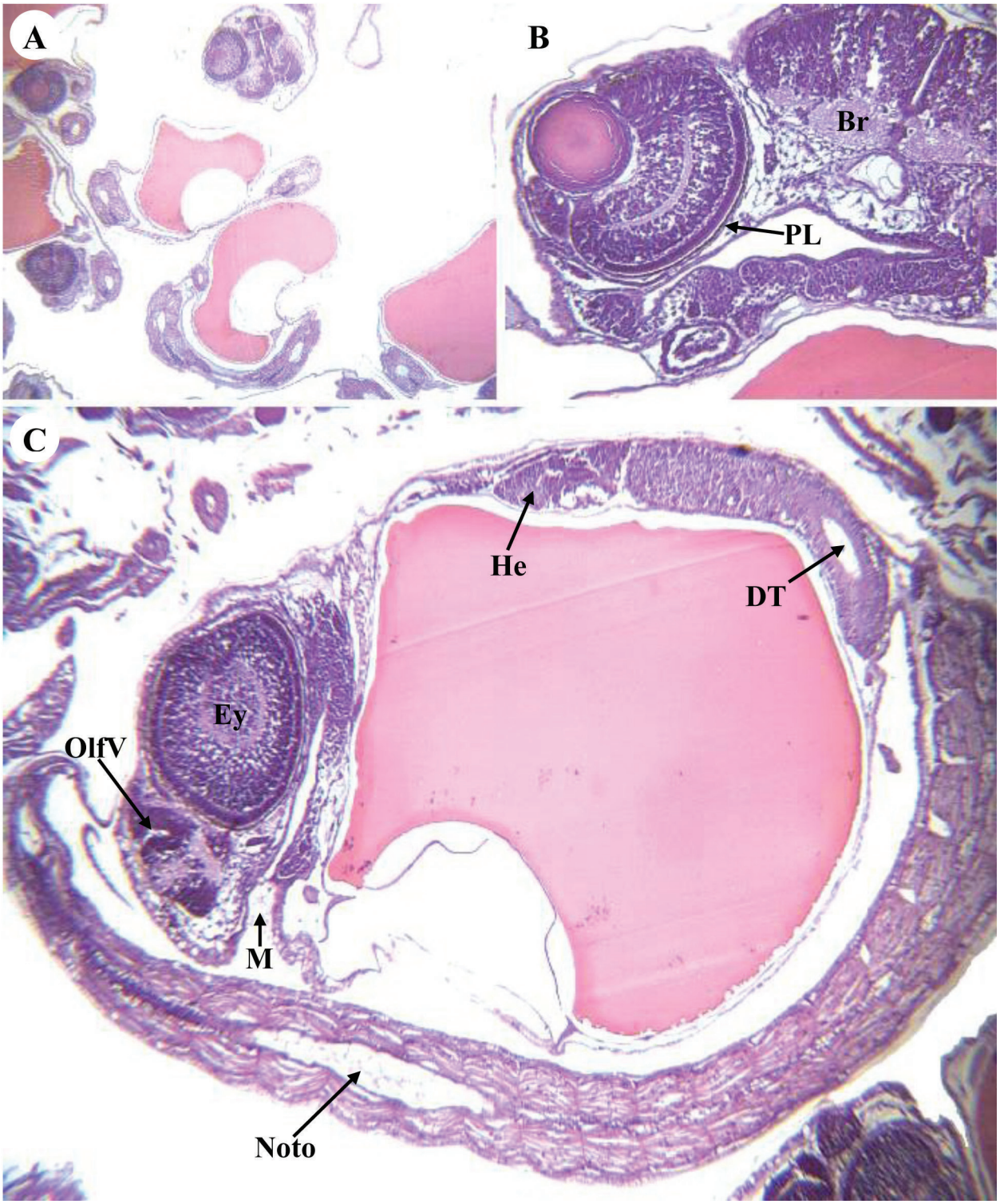

Figure 50

Heart pumping (embryonic development stage 26). *Sebastes alutus* collected 18 March 2004, 35 cm, 600 g. (A) 25×, (B) 100×, and (C) 100×.

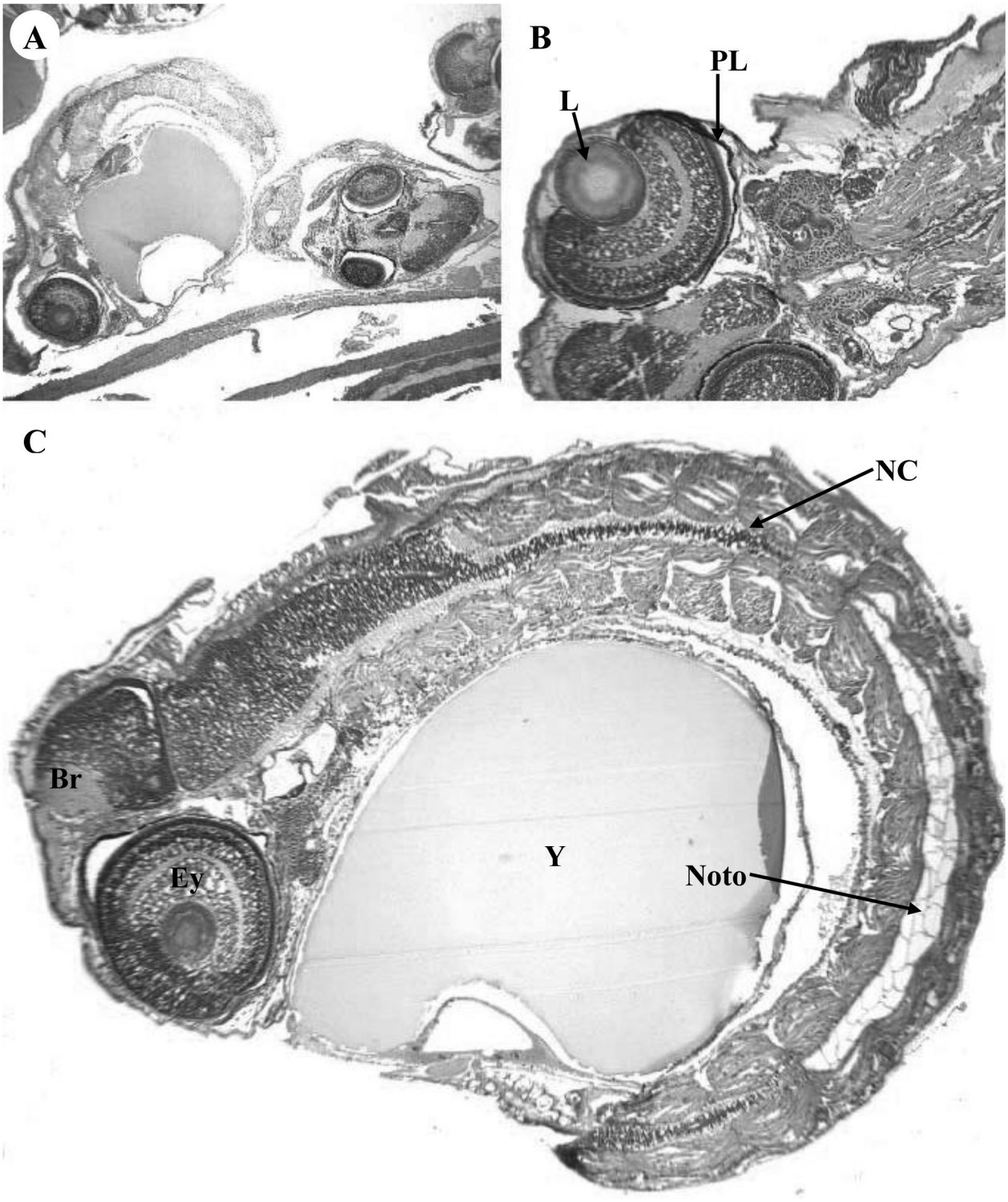

Figure 51

Lens becomes transparent (embryonic development stage 27). *Sebastes alutus* collected 25 March 2004, 38 cm, 700 g. (A) 25×, (B) 100×, and (C) 100×.

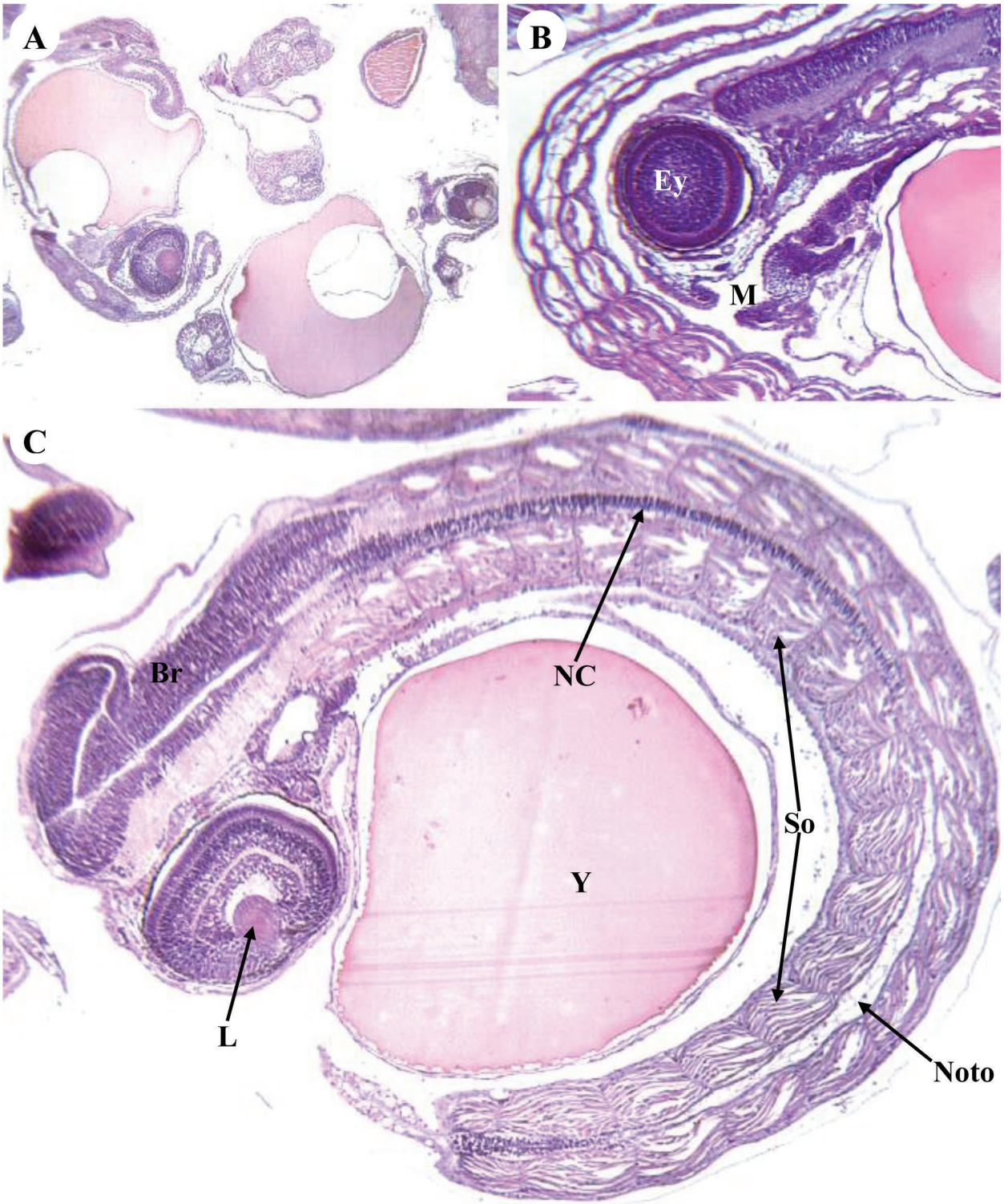

Figure 52

Openings of mouth and anus (embryonic development stage 28). *Sebastes alutus* collected 17 March 2004, 38 cm, 620 g. (A) 25×, (B) 50×, and (C) 100×.

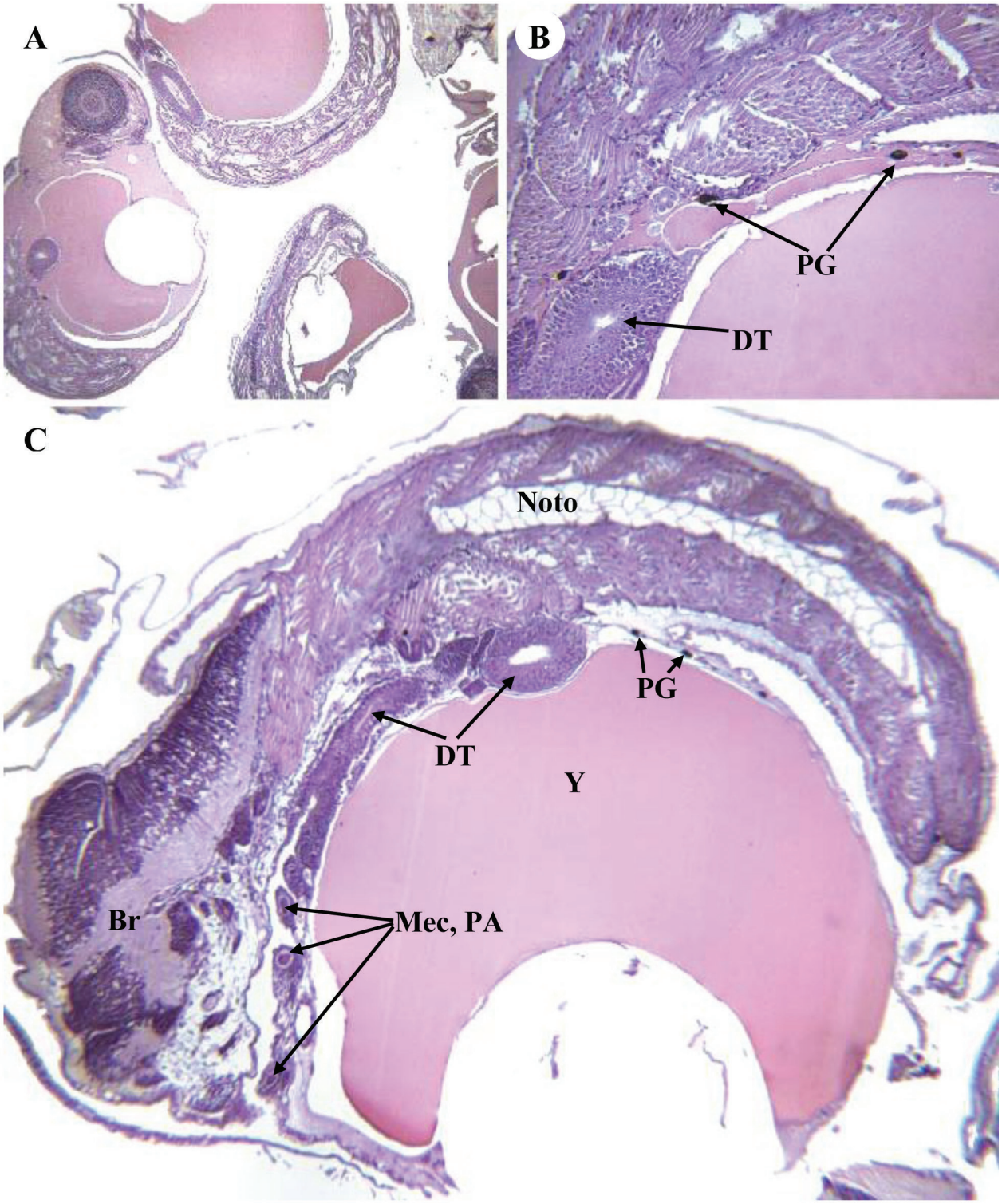

Figure 53

Peritoneal wall pigmentation (embryonic development stage 29). *Sebastes alutus* collected 22 March 2004, 39 cm, 700 g. (A) 25×, (B) 160×, and (C) 100×.

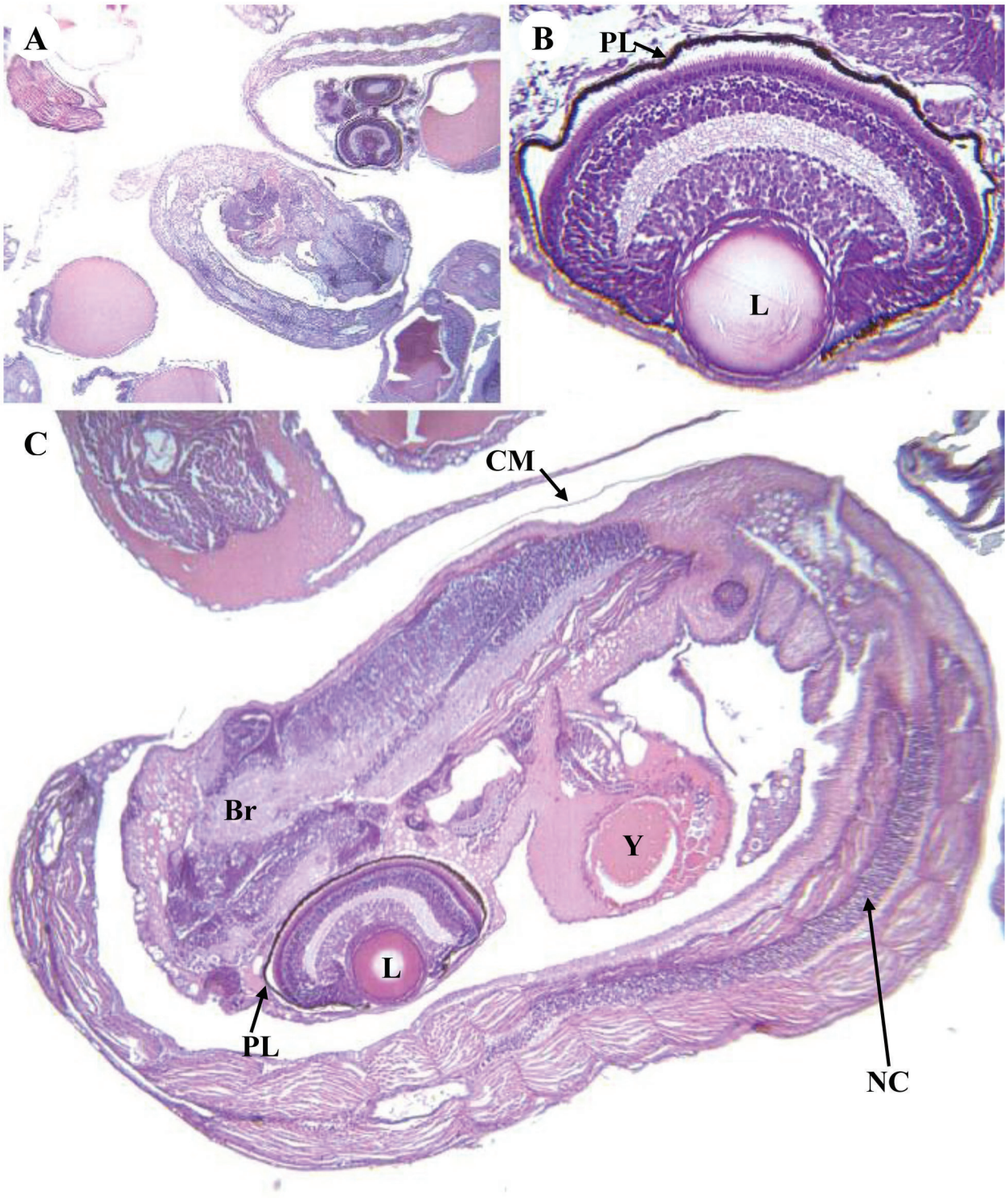

Figure 54

Depletion of yolk (embryonic development stage 30). *Sebastes alutus* collected 17 March 2004, 43 cm, 1000 g. (A) 25×, (B) 200×, and (C) 100×.

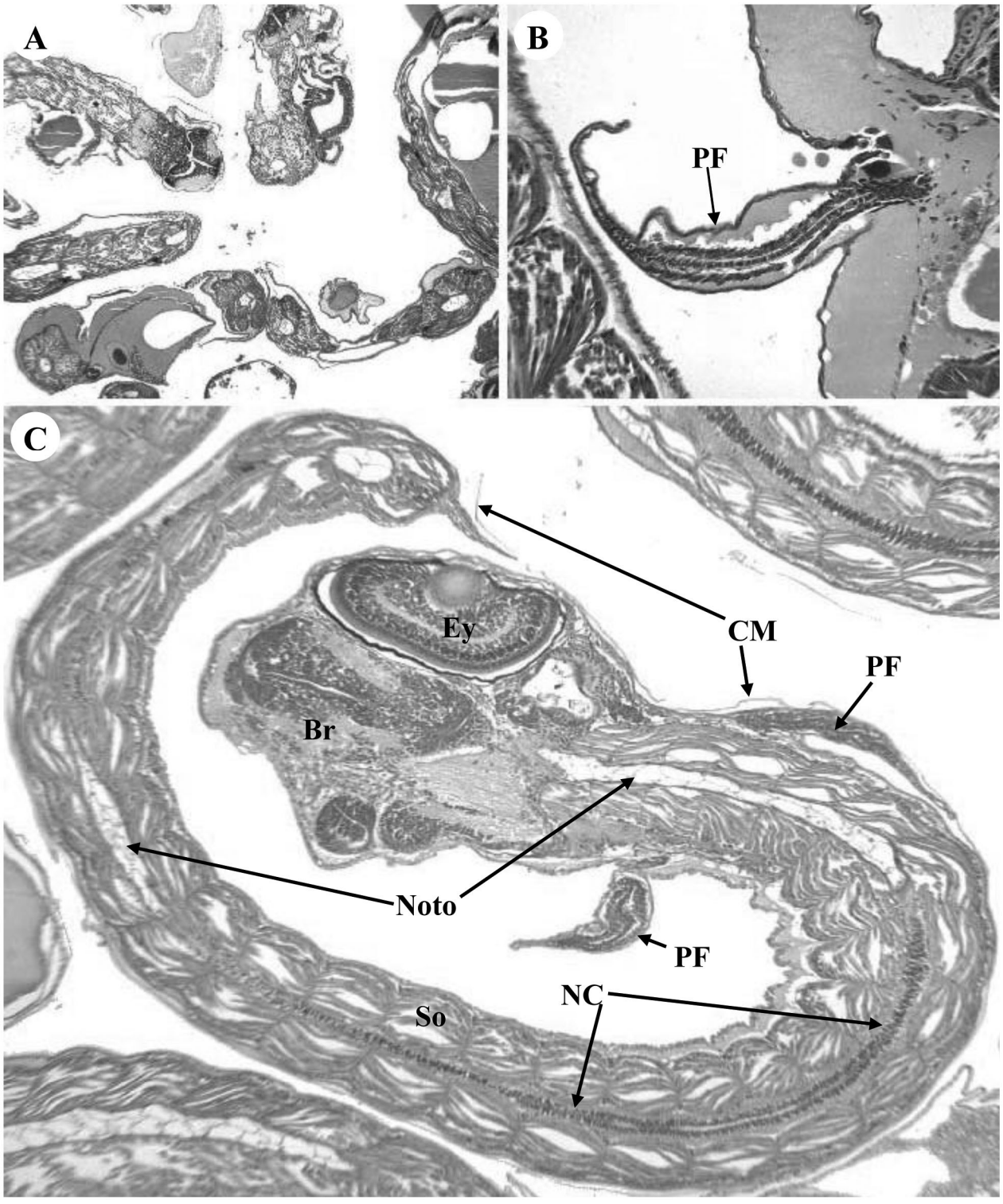

Figure 55

Prehatching (embryonic development stage 31). *Sebastes alutus* collected 17 March 2004, 42 cm, 930 g. (A) 25×, (B) 160×, and (C) 100×.

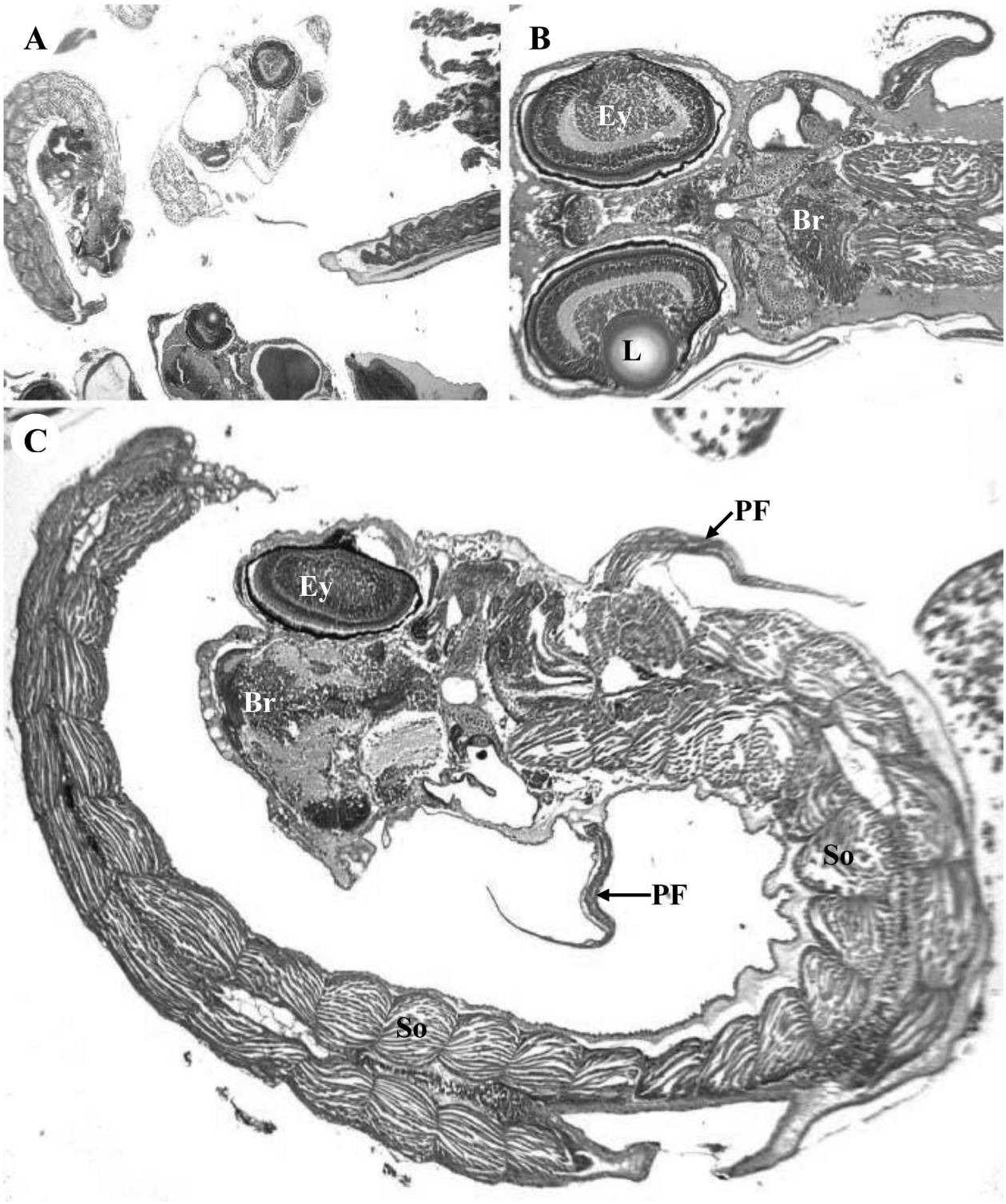

Figure 56

Hatching (embryonic development stage 32). *Sebastes alutus* collected 2 April 2004, 42 cm, 860 g. (A) 25×, (B) 100×, and (C) 100×.

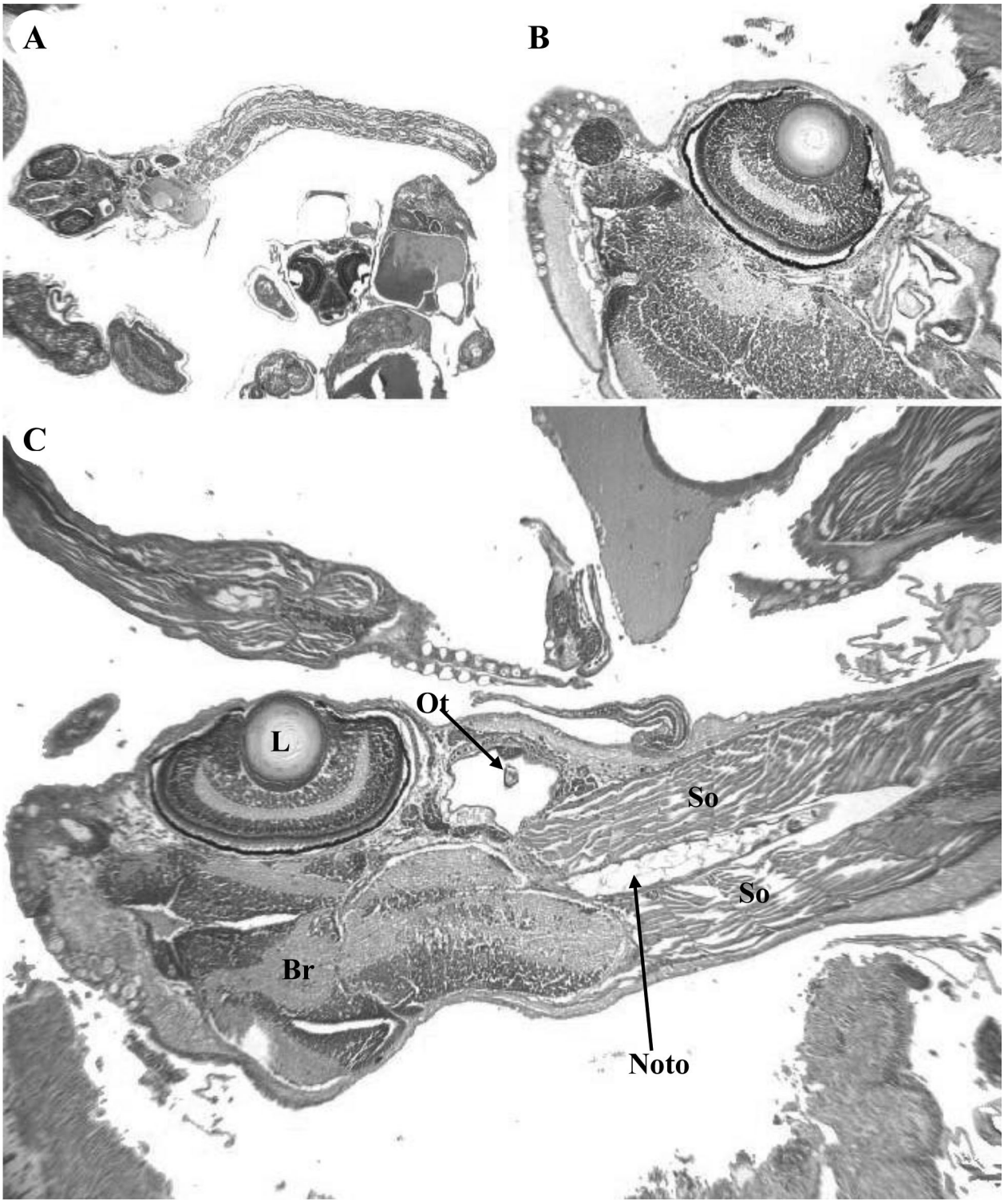

Figure 57

Newborn larva (embryonic development stage 33). *Sebastes alutus* collected 17 March 2004, 47cm, 1380g. (A) 25×, (B) 100×, and (C) 100×.

Follicular atresia

Oocytes or embryos of *Sebastes* at any stage of their development can undergo a process of resorption, which is known as follicular atresia (Saidapur, 1978; Guraya, 1979; Agarwal, 1996). Atresia is always present in postovulatory follicles, but is also observed in previtellogenic and vitellogenic follicles. Resorption of embryos may also occur at any stage of development. Atresia may also occur in preovulatory follicles; atresia serves to limit the number of oocytes that progress to the next developmental stage to a number that can be supported by the fish (Agarwal, 1996). Various other physical, environmental, and health stressors will also affect the rate of atresia (Hunter and Macewicz, 1985; Schwartz et al., 2006). Bretschneider and Duyvene de Wit (1947), working with *Rhodeus amarus*, have defined the characteristics for four stages of atretic follicles, which have been used by other researchers for other species (Lambert, 1970; Hunter and Macewicz, 1985; McDermott, 1994). Atresia in the genus *Sebastes* takes place in a similar fashion, so their original description is closely followed here.

Stage α (Figs. 58–59)

Changes in oocyte shape and size, along with increasing size of the granulosa (GR) cells, indicate the onset of atresia. Atresia commences with the contraction of the cytoplasm and continues until the entire cytoplasm and nucleus are resorbed. The vitelline membrane dissolves and the enlarging granulosa cells begin penetrating into the oocyte, phagocitizing the yolk and nucleoplasm. In unyolked oocytes and embryos, the process is similar – only the elements to be phagocitized differ. All processes of alpha stage resorption result in an empty follicle. The alpha stage could be further subdivided by whether its origin was yolked or unyolked oocytes, or an embryo.

Stage β (Fig. 59)

All the cytoplasm and yolk have been resorbed and the entire cavity is filled by granulosa cells that are increasing in size and number. The resulting structure is much smaller and more compact than the alpha stage, with noticeable disorganization in the granulosa cell layer. Large intercellular cavities are occasionally observed. At this stage, it is not possible to determine whether an oocyte started as yolked or unyolked.

Stage γ (Fig 59)

The gamma stage follicle is much smaller than the previous stages, possessing a bright red hue (H&E) resulting from the possible disintegration of the granulosa cells. Follicle cells in the gamma stage in *Sebastes* are rare compared to the other stages.

Stage δ (Fig. 59)

The size of the atretic follicles continues to shrink in the delta stage until all that remains are small groups of granulosa cells with a characteristic dark orange-brown pigment (H&E).

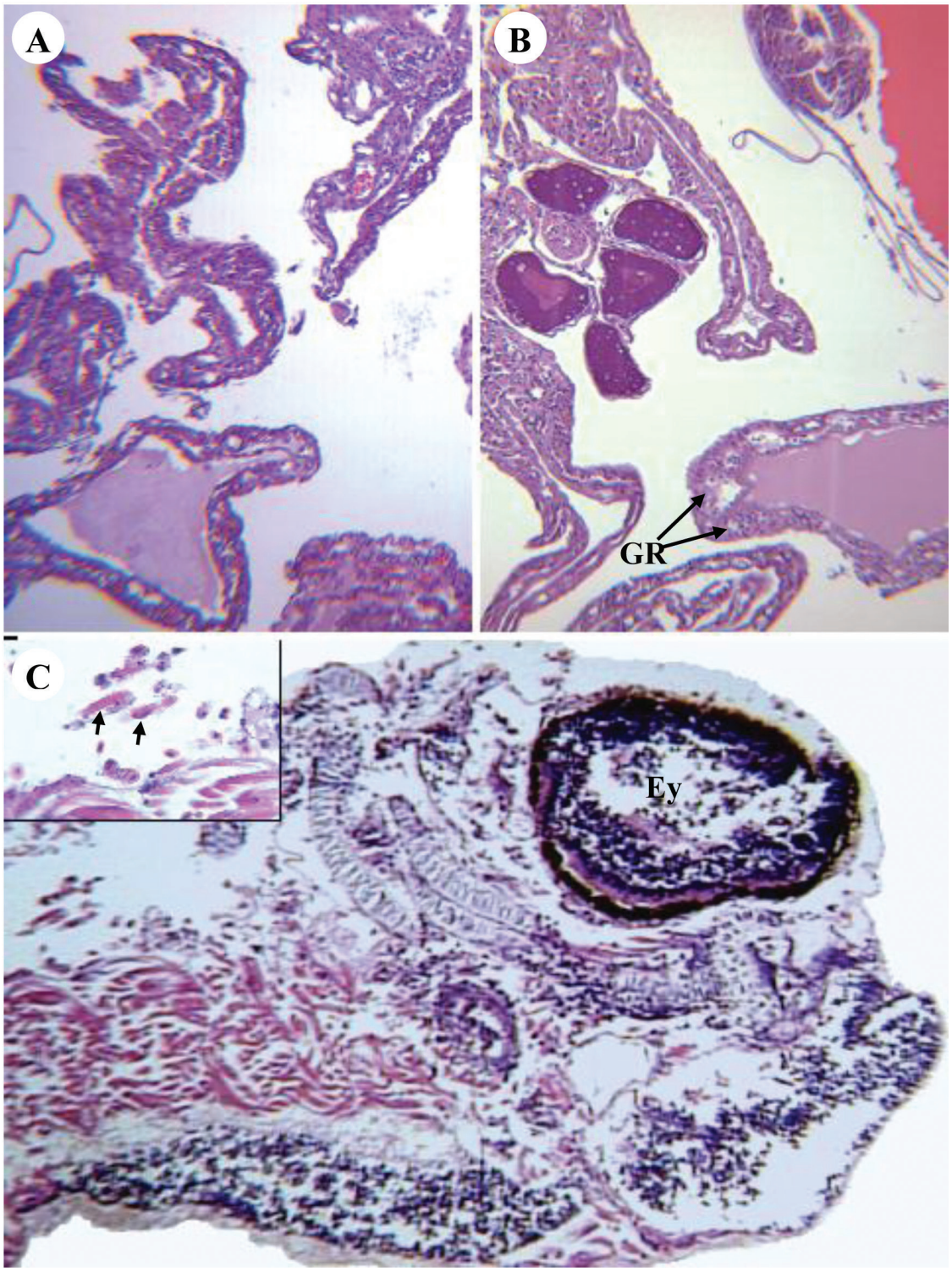

Figure 58

Alpha atresia (stage α) of oocytes and embryos of *Sebastes proriger* (inset shows cells phagocytizing the loose muscle of somites indicated by arrows). Collected 15 July 1995. (A) yolked oocytes (100×), (B) unyolked oocyte (100×), and (C) embryo (160×).

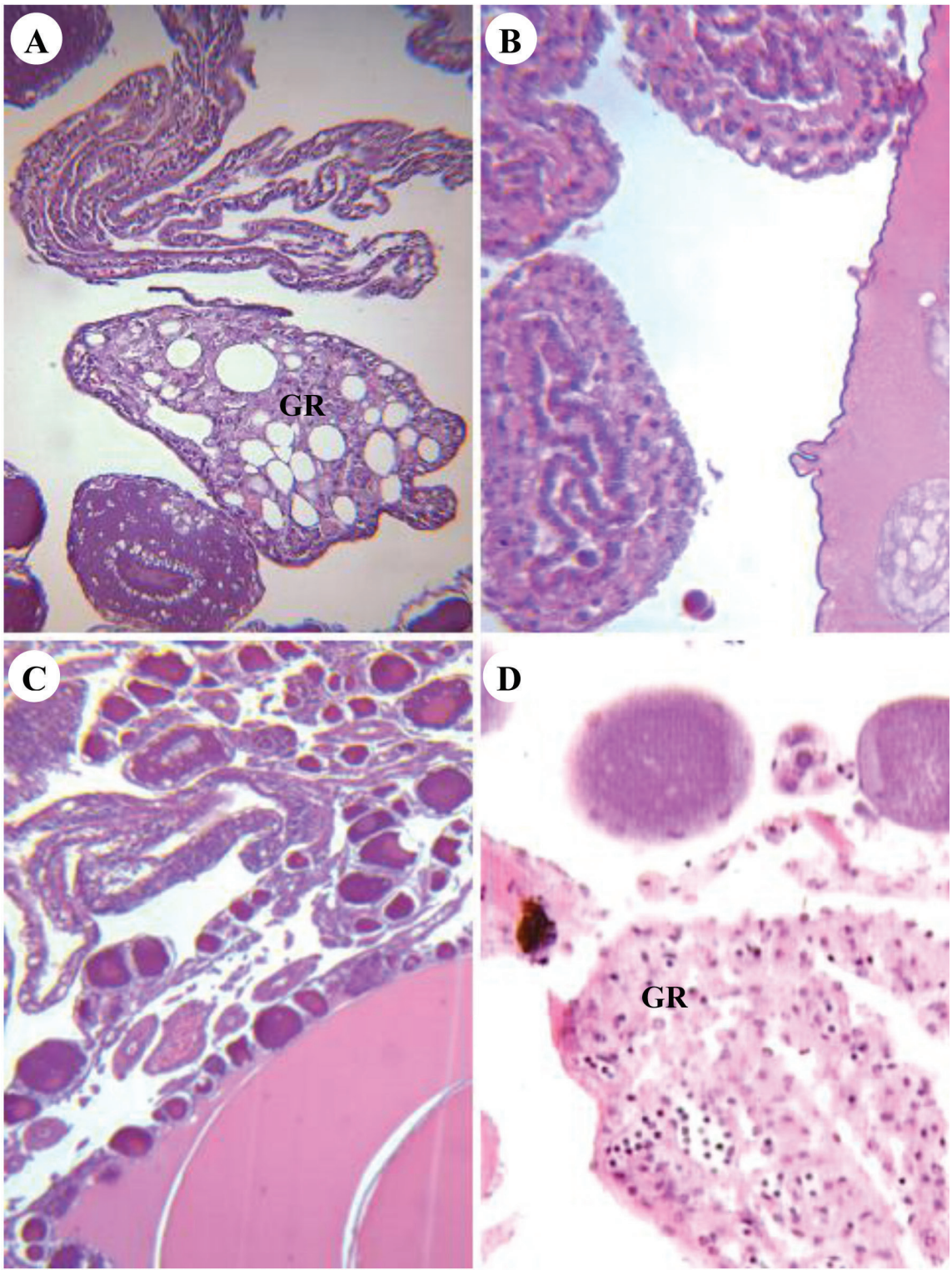

Figure 59

(A) Alpha atresia (stage α), (B) Beta atresia (stage β), (C) Gamma atresia (stage γ), and (D) Delta atresia (stage δ) yolked oocytes of *Sebastes proriger* (100×). Collected 15 July 1995.

Summary

Rockfishes of the genus *Sebastes* are known for being live bearers and long lived. Male spawning condition anticipates female maturity and during copulation, males transfer sperm to females via an intromittent organ. Females must then "preserve" transferred sperm (less the flagellum) until oocytes mature and are fertilized within the female. Females in turn produce large broods that may be released as a single spawn or in multiple spawns (Moser 1967a; MacGregor, 1970; Love, 1990; Takano et al. 1991). However, the number or frequency of annual broods may be species and age specific, as well as environmentally influenced (Love et al., 1990).

Whether that environmental influence is food availability or temperature remains to be determined. Moser (1967a), MacGregor (1970), and Love et al. (1990) confirmed in separate studies that many of the same examined species, including *S. elongatus,* in southern California were multiple brooders. However, Echeverria (1987) determined that in central California, only one of the previously examined species (*S. paucispinis*) was a multiple spawner and brooder. Still further north, Leaman (as cited in Love et al. 1990) did not find multiple brooders in any rockfish species from British Columbia. Our observations further indicate that *S. elongatus* is a single brooder in our area of study as we did not observe any secondary ova undergoing vitellogenesis during embryo development and maturation (Takano et al., 1991).

Gross development phases for both the testes and ovary are described. They are fewer in number (5) than histological phases and are therefore, less precise in reflecting gonad status. Accuracy of this method of classification, especially ovarian development, has historically been a problem (Westrheim, 1975; Chilton, 2007). Immature phases are often difficult to differentiate from late spent or resting phases and misclassifications sometimes occurred. To stage female maturity without histological confirmation, gross observations should be made during the time of parturition (late spring) since no misclassifications were made of fish sampled at these times (Westrheim, 1975; Chilton, 2007).

This study suggests that male and female reproductive biology of rockfish species are similar regardless of the number of broods produced annually. The presented phases are similar to previous rockfish gonad classifications with the exception of the addition of a regenerative phase. It is clear that although an organ may be spent, regenerative processes must occur for a subsequent spawning event, and thus the testes and ovaries are not resting. As a result, our current classification scheme generally agrees with that presented by Brown-Peterson et al. (2011) who recently called for standardization of terminology for describing reproductive development in fishes. In particular, our study appears to complement the Brown-Peterson reproductive scheme for alternative reproductive strategies (Fig. 60).

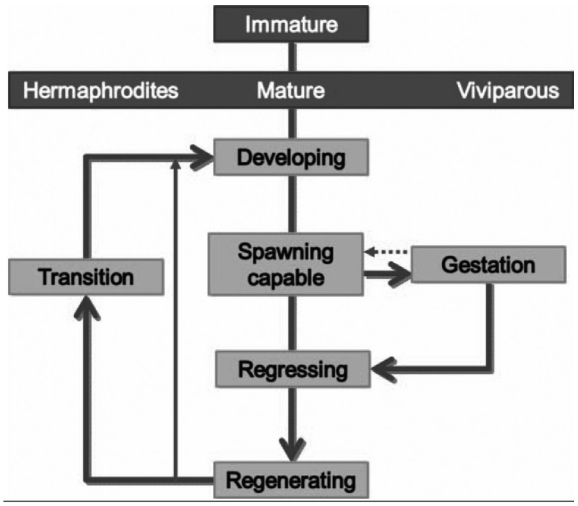

Figure 60

Modification of the reproductive phases of fishes to accommodate species with alternate reproductive strategies. The new transition phase applies to sequential hermaphrodites, and the new gestation phase applies to livebearers. Livebearers that produce more than one batch of embryos during the reproductive season cycle between the spawning capable phase as oocytes grow and the gestation phase as embryogenesis proceeds (dashed arrow). With permission from from Brown-Peterson et al. (2011).

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Text must be typed in 12 point Times New Roman font throughout. A brief introduction should portray the broad significance of the paper; the remainder of the paper should be divided into the following sections: **Materials and methods, Results, Discussion**, **Conclusions**, and **Acknowledgments**. Headings within each section must be short, reflect a logical sequence, and follow the rules of multiple subdivision (i.e. there can be no subdivision without at least two items). The entire text should be intelligible to interdisciplinary readers; therefore, all acronyms, abbreviations, and technical terms should be spelled out the first time they are mentioned. The scientific names of species must be written out the first time they are mentioned; subsequent mention of scientific names may be abbreviated. For general style, follow the *Style Manual* (2008, 30th ed.) published by the U.S. Government Printing Office and *Scientific Style and Format: the CSE Manual for Authors, Editors, and Publishers* (2006, 7th ed.) published by the Council of Science Editors. For scientific nomenclature, use the current edition of the American Fisheries Society's *Common and Scientific Name of Fishes from the United States, Canada, and Mexico*. For species not found in the above and for more recent changes in nomenclature, use the Integrated Taxonomic Information System (available at http://itis.gov/), or, secondarily, the California Academy of Sciences' *Catalog of Fishes* (available at http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp) for species names not included in ITIS. Dates should be written as follows: 11 November 2010. Measurements should be expressed in metric units, e.g., metric tons as (t); if other units of measurement are used, please make this fact explicit to the reader. The numeral one (1) should be typed as a one, not as a lower-case el (l). Write out the numbers zero through nine unless they form part of measurement units (e.g., nine fish but 9 mm). Refrain from using the shorthand slash (/), an ambiguous symbol, in the general text.

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