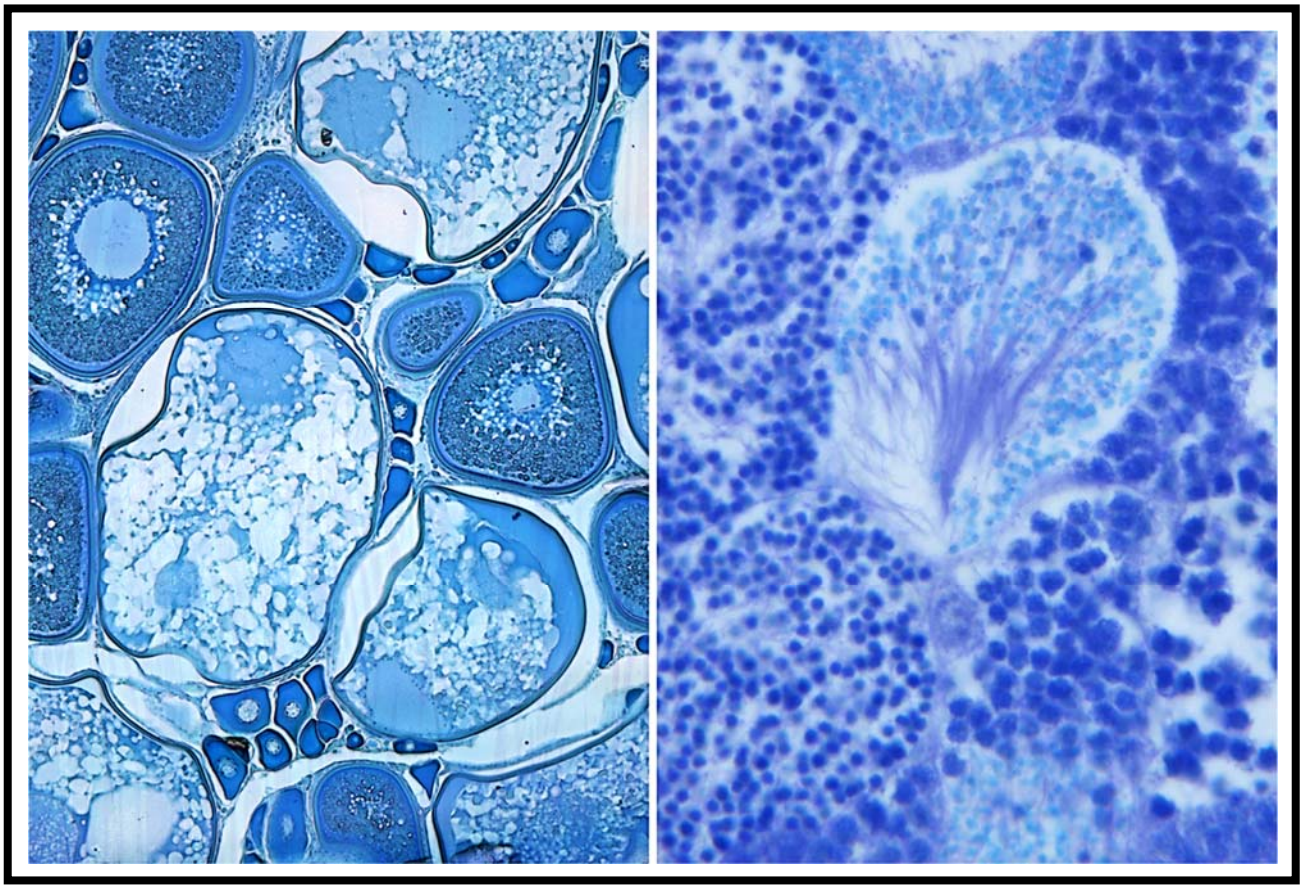
The Jungle Histology Atlas of Gonad Stages in Coral-Reef Fishes

Second Edition

Ken Longenecker and Ross Langston



Honolulu, Hawaii

May 2018

COVER

Gonad sections produced using “Jungle Histology” methods. Left: mature female *Acanthurus lineatus* (100X). Right: mature male *Caesio cuning* (1000X). Images: Ross Langston.

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

PREFACE TO THE SECOND EDITION

The first edition of this atlas, published in December 2016, was created to provide a simple pictorial guide that could be used by students and resource managers who wish to perform histology-based reproductive analyses for reef fishes. That atlas was created in response to a suggestion from participants in our first Jungle Histology workshop held in Kavieng, Papua New Guinea in 2015. Since publishing the first edition, we have amassed more images and received feedback from participants in two additional Jungle Histology workshops (Guam and American Samoa) conducted during 2017. The second edition incorporates that feedback and builds on the first edition as follows:

- A section on specimen dissection and gonad preservation has been added
- We have included generalized descriptions of gonad anatomy and gamete development
- Additional images have been added to illustrate species-level differences in gonad stages
- The size of existing image collages has been increased to show greater detail
- Image brightness and contrast has been adjusted for better printing
- Line-art figures have been added to better illustrate key features of gonad anatomy and gamete stages

ACKNOWLEDGMENTS

Production of this atlas was supported by the Saltonstall-Kennedy Grant Program, project number NA16NMF4270264. The images featured in this atlas are products of past research projects funded by the Hawaii Coral Reef Initiative, the Dingell-Johnson Sport Fish Restoration program, a private foundation wishing to remain anonymous, and the Saltonstall-Kennedy Grant Program. We thank the participants in the first Jungle Histology workshop (Matthew Crane, Terry Donaldson, Erik Franklin, and Mildred Kelokelo) for suggesting that an atlas would be a valuable tool for future training and for producing some of the gonad sections used in this atlas. The Nago Island Mariculture and Research Facility (in New Ireland Province, Papua New Guinea) provided in-kind support for the first Jungle Histology workshop, and Tapas Potuku was instrumental in obtaining the specimens analysed by workshop participants. Our thanks to the residents of Kamiali Wildlife Management Area (Morobe Province, Papua New Guinea) for collecting many of the other specimens used in the production of this atlas. We also thank our Kamiali research partners Holly Bolick, Utula Kondio, and Mara Mulrooney who helped produce many of the gonad sections used in this atlas. Finally, we thank the many participants in each of the Guam and American Samoa Jungle Histology workshops for “test-driving” the first edition and a working draft of the second edition, respectively. Figure 21 courtesy of Bishop Museum.

INTRODUCTION

Purpose

This atlas is intended to supplement the Jungle Histology online training series, available at: http://pbs.bishopmuseum.org/pacificfishes/modules.html. Jungle Histology is our rapid, low-cost, portable, histology-based approach to the reproductive analysis of coral-reef fishes. Importantly, the methods are easy to learn and require minimal research infrastructure. Thus, we think that teaching Jungle Histology methods to resource managers from Pacific islands, where many of the world's coral reefs are located but that often lack reproductive-research capacity, will help address fishery management's woefully limited knowledge about reef-fish reproduction. Toward that end, in 2015 we hosted the first Jungle Histology training workshop at the Nago Island Mariculture and Research Facility, Kavieng, New Ireland Province, Papua New Guinea. Participants at that workshop initially had trouble evaluating gamete maturity, and suggested that a pictorial guide to fish gonads and gamete development would be a helpful training tool. This atlas is our attempt at addressing that need. We hope it will be useful to a broad range of fish biologists.

Organization

The Jungle Histology approach is pragmatic. So is this atlas. The atlas is divided into eleven sections.

The following, second, section addresses specimen dissection, retrieval, and preservation of gonads. Those who are already familiar with the process can skip this section.

The third and fourth sections describe the anatomy of female and male gonads, respectively, and provide information that should allow the reader to evaluate whether or not a prepared gonad section came from a mature individual. Each of these two sections is further subdivided into subsections. Each subsection (oocyte stages for females, maturity stages for males) begins with the name of the stage, line drawings and close-up image(s) of the stage, a description of the stage with diagnostic characters in bold font and the most-salient feature(s) in underlined bold font. Descriptions may be followed by notes providing additional or clarifying information. Most subsections include a set of images, from a variety of species, showing additional examples of the gonad stage.

The fifth section shows examples of undifferentiated gonads, which may be found in small specimens. The sex of these immature individuals cannot be determined by Jungle Histology methods.

The sixth section (Other Features) presents structures that may be present in reef-fish gonads. Perhaps more important than the short description following each image is the subsequent note. These notes explain what the feature may indicate. In some cases, the feature may suggest sex change.

Given that sex change is relatively common among reef fishes, and that many of the other features presented in the sixth section suggest sex change, the seventh section (Ovotestis) focuses on hermaphroditism. It shows a transitional gonads, which, when common in a population, may indicate sex change.

The eighth section examines non-gonadal tissues and structures. When excising gonads from a specimen, other organs or tissues may be attached to (or may be mistaken for) gonads. These non-target structures, when included on a prepared tissue section, may confuse those new to the art and science of histology. We present tissues and organs that commonly appear on tissue sections,

indicate gonadal structures with which they may be confused, and provide characters to help differentiate between gonads and non-target structures.

The ninth section focuses on making prepared slides easier to interpret. This includes a subsection on hematoxylin and eosin (H & E) staining. An H & E protocol is not taught during Jungle Histology training because it is more expensive, complicated, and time-consuming than the Jungle Histology approach of staining with toluidine blue. However, compared to the toluidine-blue method, H & E staining makes it easier to distinguish between immature and mature specimens (especially females with oocytes at the cusp of vitellogenesis). This subsection shows tissues stained with H & E to illustrate the relative ease with which immature and mature individuals can be distinguished. The following subsection presents an H & E staining protocol for those who may be interested in more-advanced staining techniques.

The tenth section is a glossary. Although we have tried to keep jargon to a minimum, some specialized terminology cannot be avoided. We present simplified definitions for words necessary to make the most of this atlas. The same set of terms should prepare the user for reading scientific literature focused on fish reproduction.

The final section is a very limited bibliography. We provide the citations to our own publications resulting from the Jungle Histology approach. We also provide references for publications that form the basis of our understanding of fish-gonad histology.

Although evaluation of gamete stages does not require a detailed understanding of reproductive physiology, we recognize that some users (*e.g.*, new graduate students working in the field of histology) may appreciate a succinct explanation of key physiological processes. For this reason, we have included brief explanations of those processes throughout the text. Rest assured that this information is *not* required to stage gonad sections successfully.

Scoring Gonad Sections

An Iterative Process

Initial attempts at assigning a developmental or maturity stage can be frustrating. Even experienced histologists will vacillate, especially when judging oocyte stages.

We recommend examining all prepared slides at least twice. A preliminary evaluation can be done as each batch of slides is processed. By the time all slides have been prepared and examined, the investigator should have a reasonably trustworthy sense of the visual characteristics of each stage.

The second evaluation should be done in sequential order, by specimen number. At this point, the investigator should be able to assign, with sufficient confidence, maturity and developmental stages.

Alternatively, two trained observers can evaluate the sections independently. Specimens that are identically scored can be entered into the data record without further deliberation. Those for which there is disagreement should be evaluated a third time with both observers present. In these cases, they should carefully evaluate the specimen using the written criteria/descriptions for each stage. Once a consensus is reached, the data can be entered into the record.

Size Matters

The following descriptions of oocyte stages indicate that the diameter of each stage is usually at least two times that of the previous stage. Investigators can take advantage of this phenomenon to help assign oocyte stages.

The size range of each oocyte stage is species specific. Therefore, the investigator will need to measure the diameter of a series of confidently scored oocytes for each stage. These measurements can be made with an ocular micrometer. Based upon at least 10 measurements for each stage, the investigator can then calculate the mean diameter, and range, for successive oocyte stages. This information can then be used to more-confidently score other ovary sections.

SAMPLING GONADS

Whole Specimens

Tissue decomposition is the histologist's biggest enemy. As the saying goes, "garbage in, garbage out." A rotten gonad will yield virtually useless histological preparations. The degradation may not be obvious until microscopic examination (*i.e.*, after doing the time-consuming work of fixing, dehydrating, embedding, sectioning, and staining). The histologist can avoid this disappointment by proper handling of whole specimens. Because tissue decomposition begins immediately upon the death of an organism, whole specimens should be frozen shortly after capture and kept frozen until gonads are excised. If freezing is not an option, gonads should be excised shortly after capture. Either way, gonads should be fixed immediately upon removal from the specimen.

Dissection

Removing the gonads is usually a relatively straightforward process because the internal anatomy of most reef fishes is remarkably similar (Figure 1). The gonads are typically sandwiched between the digestive organs and the gas bladder (when present), and held closely to the gas bladder by connective tissue. The gonads connect to the vent at the posterior end of the visceral cavity. Figure 2 illustrates how to expose the gonads of fishes with the typical body plan.

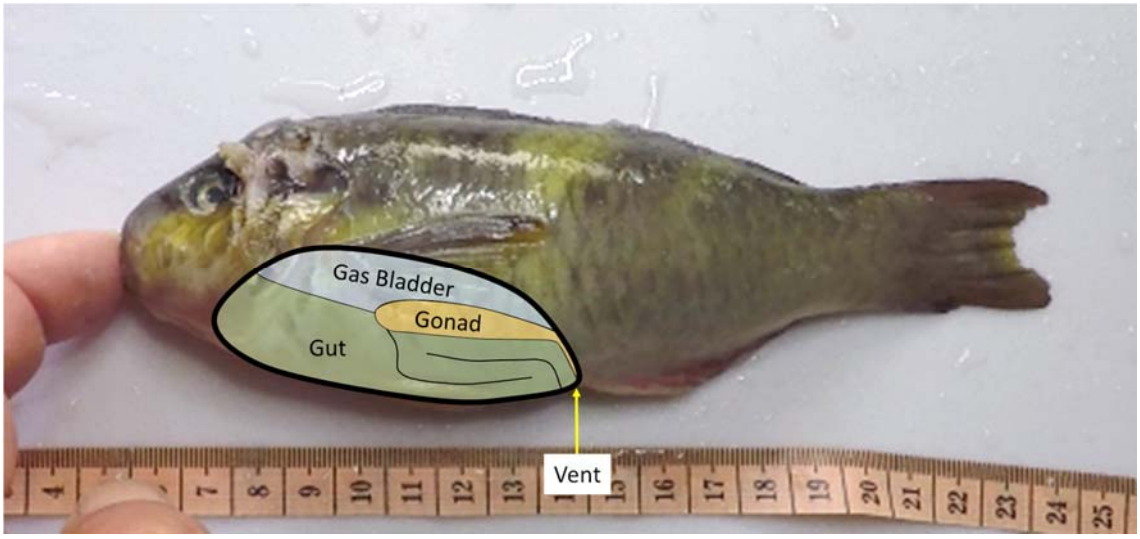

Figure 1. Idealized diagram of gonad location in a typical coral-reef fish.

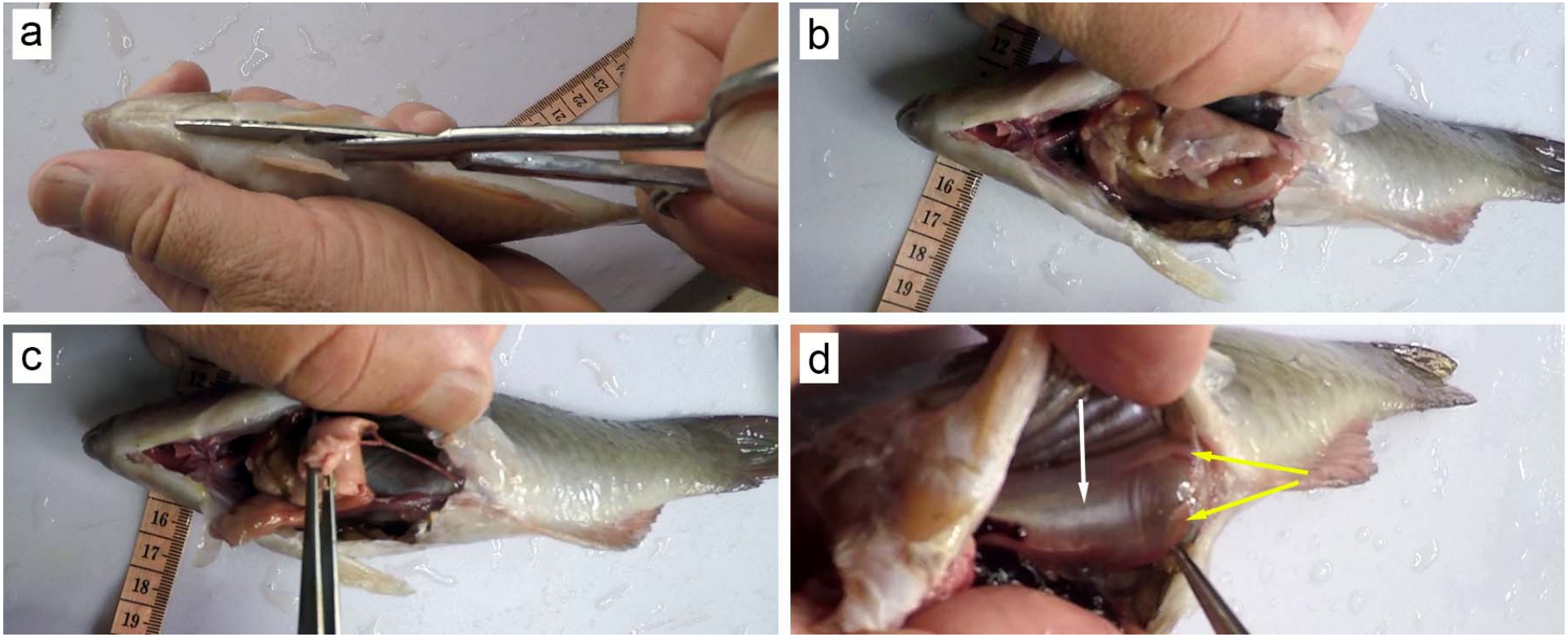

Figure 2. Method for exposing the gonads in a typical coral-reef fish. (a) Orient the fish ventral side up, Insert one tip of scissors into the vent, and make a shallow cut forward to the head (pull up lightly with the scissors and keep them parallel with the belly wall to avoid cutting the digestive organs). (b) Pull the resulting belly flaps laterally to expose the digestive organs. (c) Pull the digestive organs toward the head. (d) The paired gonads (yellow arrows) are attached by connective tissue to the gas bladder (white arrow).

The surgeonfishes have a modified body plan; their gonads are located in the ventral part of the visceral cavity, which extends posterior to the vent (Figure 3). The approach illustrated in Figure 2 would only expose the gut. Figure 4 illustrates a convenient method to expose the gonads of surgeonfishes. Take note of the fat body illustrated in Figure 3; it is often mistaken for testes.

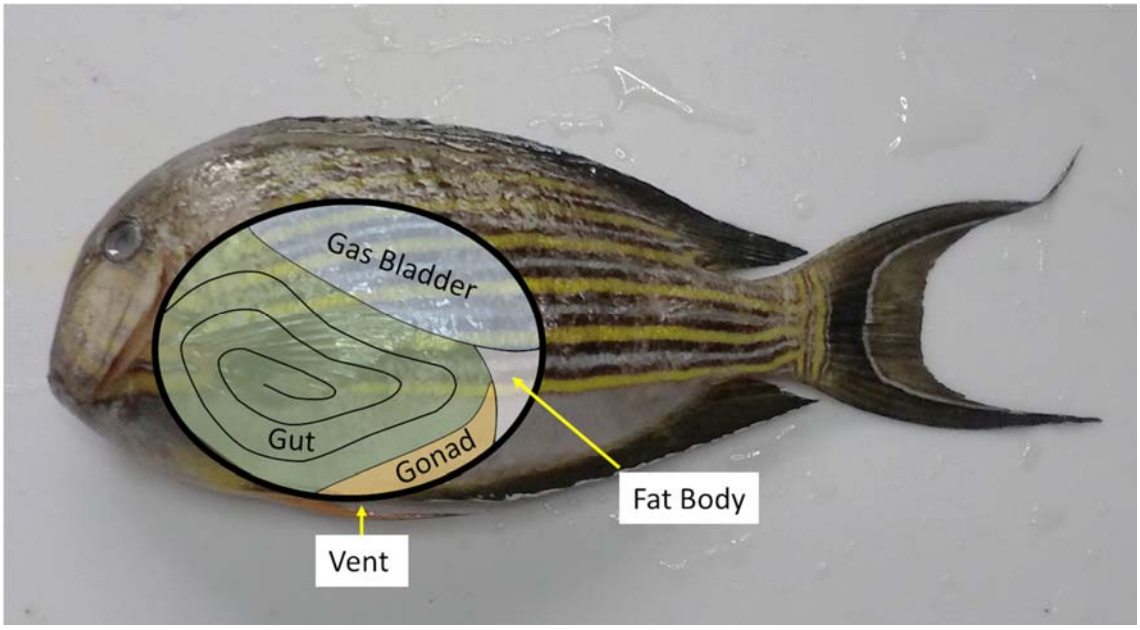

Figure 3. Idealized diagram of gonad location in a typical surgeonfish.

Processing Gonads

We reiterate that gonads should be fixed immediately upon removal from the specimen. The Jungle Histology online training series (http://pbs.bishopmuseum.org/pacificfishes/modules.html) explains how to process specimens whether or not they can be frozen. A detailed, printed version of the gonad-processing protocol is presented in Longenecker et al. (2017).

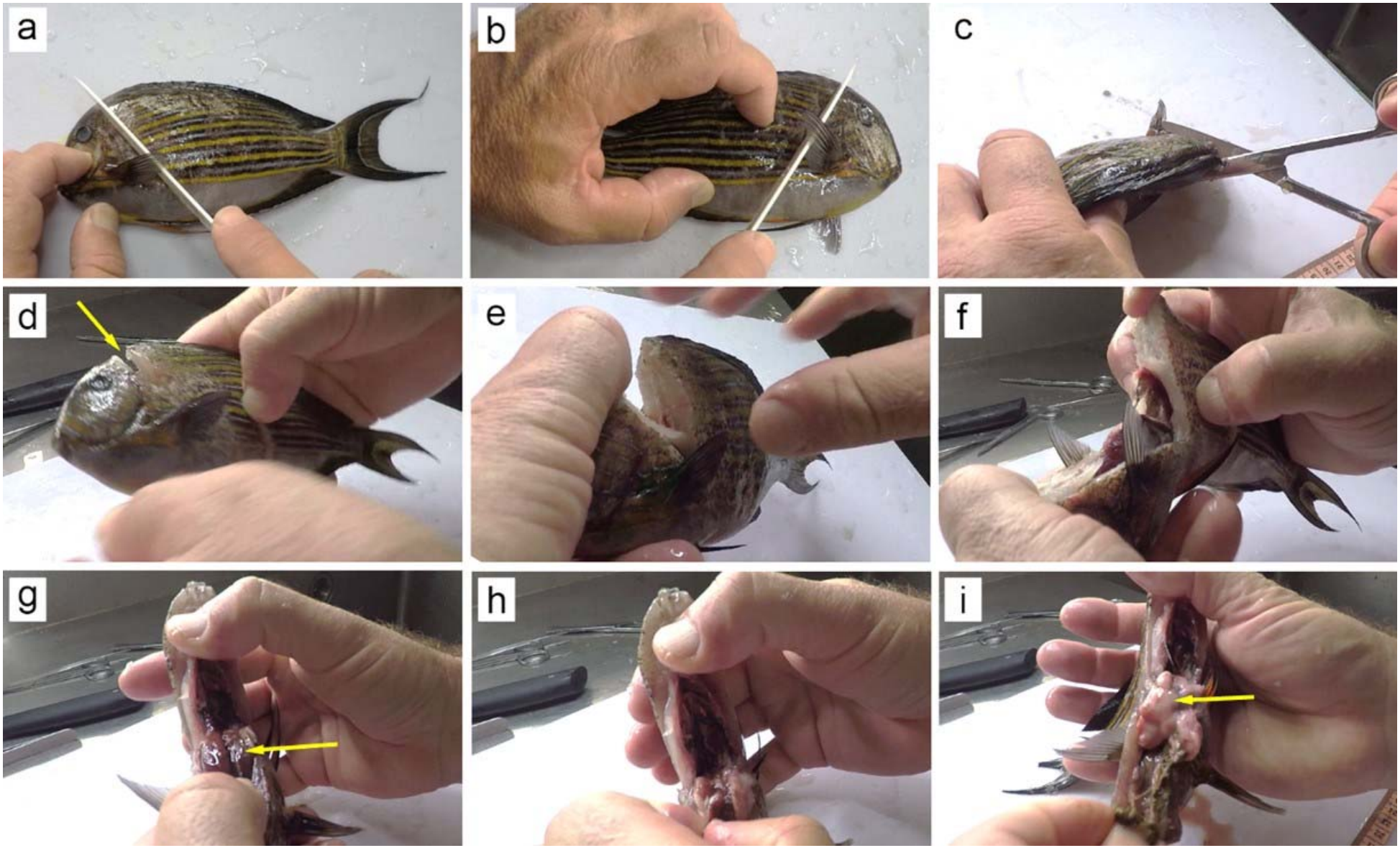
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Figure 4. Method for exposing the gonads in a typical surgeonfish. (a) Cut one side of the fish diagonally from the nape to the area between the vent and the origin of the first anal spine. The cut should be deep on the dorsal surface (reaching, but not cutting through, the spine), but barely scoring the belly wall (i.e., do not cut into the visceral cavity). (b) Make a parallel cut on the other side. (c) Use sturdy/heavy-duty scissors to complete the dorsal cuts through the vertebrae (i.e., sever the spinal column). (d) The specimen will now have a cut completely through the dorsal surface (arrow), and be scored on both sides of the belly. (e) Pull forward and down on the head to tear the belly wall along the scores made with the knife. (f) Continue pulling until the head is attached to the body only by the "hinge" between the vent and first anal spine. (g) The digestive organs (arrow) will now be exposed. (h) Pull the digestive organs toward the head. (i) The gonads (arrow) will be exposed, resting on the "hinge" between the vent and first anal spine.

FEMALES

Ovarian Anatomy

Ovaries are the gonads of females. In most fishes, the ovaries are bi-lobed, although one lobe may be much larger than the other. When sectioned transversely (cross section), they usually appear circular in shape and consist of a central **lumen** (cavity) bounded by ovarian tissue (Figure 5). This tissue consists a mixture of oogonia, **oocytes** (eggs) and stromal (supportive) tissues, including connective tissue and blood vessels. Finger-like projections, called **lamellae**, extend into the lumen. The lumen of the ovary is continuous with the gonadal duct through which mature oocytes will leave the body during spawning. When sampling an ovary, it is important to include a portion of the lumen; the most-mature oocytes will often be found within or adjacent to the lumen.

A gonad wall consisting of smooth muscle and fibrous connective tissue surrounds the ovary. The gonad wall may also contain large blood vessels. Although some authors use the thickness of the ovarian wall as an indicator of maturity, ovaries are most-often classified as mature or immature on the basis of oocyte development.

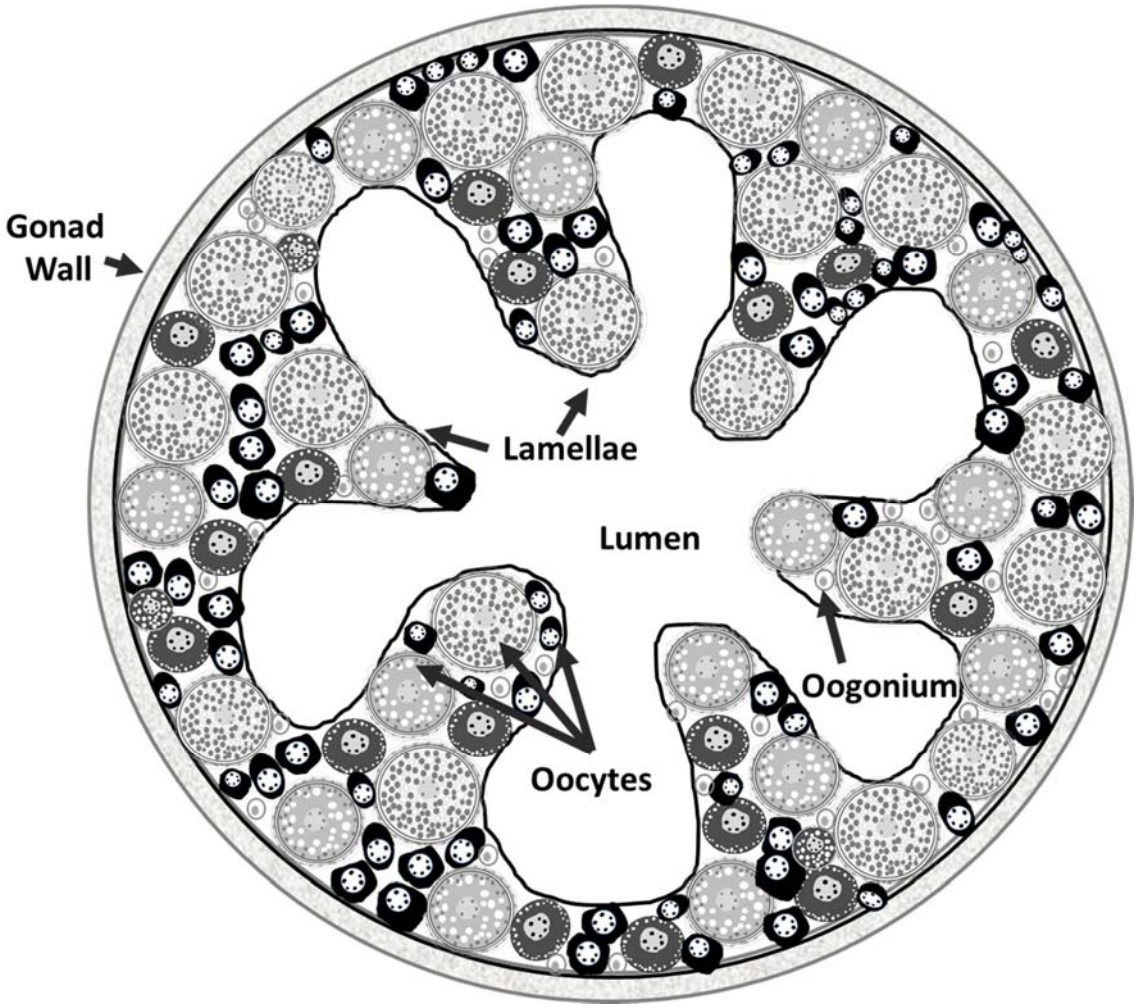


Figure 5. Idealized anatomy of a coral-reef fish ovary. This diagram shows anatomy of a mature female containing each of four oocyte stages.

Oocyte Development

Oocytes (eggs) are the gametes of females. All oocytes are derived from **oogonia**, immature reproductive cells located in the ovarian tissue. The process by which an oogonium becomes a mature oocyte (**oogenesis**) is complex, involving cell division and signals from the endocrine system. Suffice it to say that, as an oocyte matures, it grows in size and accumulates the raw materials (carbohydrates, lipids, and proteins) needed to support the future embryo. As it matures, the appearance and staining properties of the oocyte change. These changes form the basis of the classification system presented in Table 1.

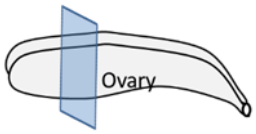
The "youngest" oocytes, (known as **primary growth stage**) are irregularly shaped (*i.e.*, angular). They also have dark-staining cytoplasm, and contain a prominent circular nucleus containing multiple nucleoli.

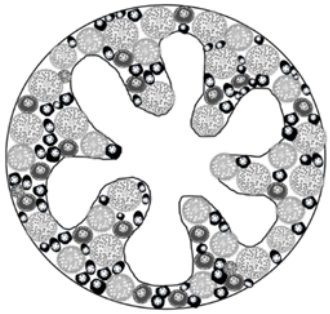
As they continue to develop, oocytes will assume a less angular shape and will begin to accumulate clear lipid (oil) droplets within the cytoplasm, marking the beginning of the **yolk vesicle stage**. These oil droplets will serve as an energy source for the future embryo and, in pelagic spawners, will help ensure that the eggs are buoyant. As the oocyte continues accumulating lipid, the cytoplasm changes from dark- to lighter-staining, and the shape of the nuclear membrane changes from circular to irregular. The oocyte also develops a conspicuous covering or **follicle** composed of epithelial cells (granulosa and thecal cells). The follicle is responsible for nurturing the oocyte and producing hormones that control oocyte development. Later-stage oocytes also develop a clear, acellular "shell" known as the **zona radiata** or zona pellucida. This membrane lies between the follicle and the cytoplasm of the oocyte and regulates the passage of materials into the oocyte.

Eventually, the oocyte will begin to accumulate **vitellin**, a type of yolk protein essential to the development of the embryo. This process is known as **vitellogensis.** Unlike lipid droplets (which do not typically stain), vitellin stains moderately with toluidine blue or eosin. Initially, the vitellin accumulates as small, uniform granules or globules at the oocyte periphery. As development progresses, these granules become equally distributed throughout

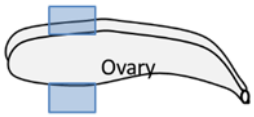
A Note about Sectioning Planes

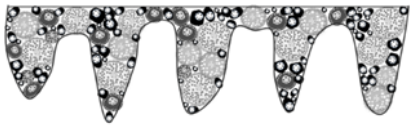
The histological appearance of gonads may differ markedly depending on sectioning plane. The sectioning plane is determined by the orientation of the cutting blade to the gonad. Traditionally, fish ovaries are sectioned **transversely** (cross section), which yields a more-or-less circular gonad section containing a central lumen (see below).

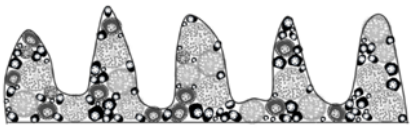




In contrast, a **longitudinal** (length-wise) cut will yield a rectangular section in which the lumen extends from left-to-right or top-to-bottom (see below).







Regardless of the sectioning plane used, a gonad can be classified as mature or immature on the basis of gamete stages present.

the cytoplasm. The presence of vitellin within *any* oocyte in the ovary indicates the onset of sexual maturity.

During the last stage, **final maturation**, the vitellin globules begin to coalesce into larger globules of different sizes. In some species, the globules form homogeneous sheets of yolk protein. Lipid droplets likewise coalesce into larger vesicles. The nucleus typically "disappears" and, in marine spawners, the oocyte increases several-fold in size as it rapidly accumulates water during hydration. On prepared slides, hydrated oocytes may have an irregular "star" shape due to uneven tissue dehydration during sample processing.

Once an oocyte fully matures, the follicle will rupture, releasing the egg into the ovarian lumen (**ovulation**). After ovulation, the oocyte then passes out of the body via a gonadal duct. The ruptured follicle is temporarily retained in the ovary and typically changes, in a matter of hours or days, from an open sac to a compacted ball of cells. For this reason, presence of **post-ovulatory-follicles** (POFs) within an ovary is evidence of recent spawning and thus sexual maturity.

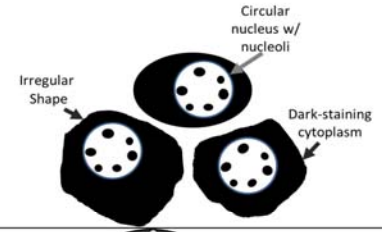
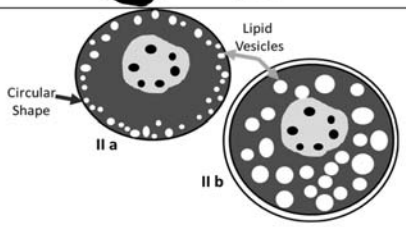
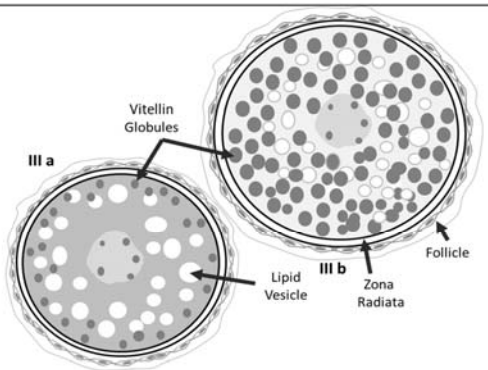
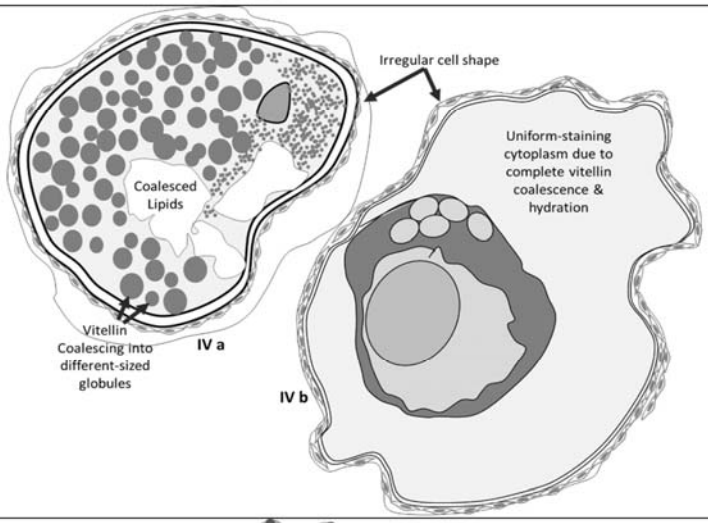
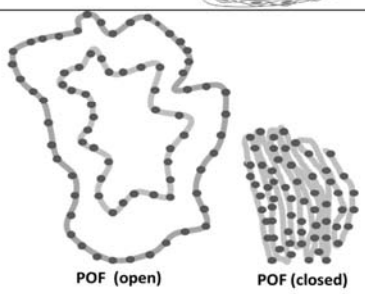
Oocyte Classification

Numerous schemes have been proposed to classify the oocyte stages of coral-reef fishes. For simplicity, we recognize only four main stages (indicated by Roman numerals I-IV) corresponding to the processes described above. These stages are summarized in Table 1 and expanded upon in subsequent sections that also include photographs of each stage. Two important notes: 1) Ovaries should be classified by the most-advanced oocyte stage present. Thus, an ovary with stage I, II, and III oocytes is classified as stage III. 2) Individuals whose ovaries contain *only* stage I or II oocytes are classified as immature (or inactive) whereas those with stage III or IV oocytes are considered mature.

Please note that, although the task of oocyte classification requires you to "lump" oocytes into discrete stages, there is a continuum of oocyte development. Thus, it is possible that an oocyte will have features intermediate between two stages. In these cases, use your best judgement and the criteria in Table 1 to make a decision. For example, an oocyte with dark-staining cytoplasm (characteristic of stage I and II) but also containing small, equally sized vitellin granules (characteristic of stage III) would be classified as stage III, because the presence of vitellin is diagnostic of stage III.

Finally, a word about detail. Although we sometimes classify stage II-IV oocytes into "early" and "late" (a & b, respectively) sub-stages in this atlas, this distinction is unnecessary if the goal of your study is simply to estimate the size-at-maturity for a fish population. In this case, you just need to decide if the specimen you are examining is immature (contains stage I-II oocytes only) or mature (contains stage III or IV oocytes, or post-ovulatory follicles). In contrast, if your goal is to document spawning periodicity, you should attempt to clearly distinguish each of the four stages and, if possible, differentiate early- and late- stage III and IV oocytes from one another because this will give you the greatest degree of sensitivity when attempting to relate oocyte development to the passage of time.

Table 1. Oocyte-stage classification.

Stage	Description	Example
I- Primary Growth	Small, **irregularly shaped** oocytes with uniform **dark-staining cytoplasm** and a prominent pale **circular nucleus containing multiple nucleoli**. Nucleus occupies >30% of cell volume. **Zona radiata absent.**	
II- Yolk Vesicle	Cell outline is **smoother** (less irregular) than Stage I. **Moderately-dark-staining** cytoplasm containing a few (IIa) or many (IIb) pale **lipid vesicles.** Nucleus becoming irregularly shaped. Zona radiata may be present in late stage (IIb).	
III- Vitellogenis	Cells with uniform **vitellin globules** which stain moderately with toluidine blue or eosin. Globules initially appear at the oocyte periphery (IIIa) then distribute evenly throughout cytoplasm (IIIb). **Cytoplasm lighter** than previous stages. **Zona radiata and follicular layer prominent.**	
IV- Maturation	Cells double-in-size when compared to previous stage. Nucleus indistinct or absent. **Vitellin coalesces into larger globules of different sizes** (IVa) or forming uniformly-staining "sheets" within cytoplasm (IVb). Lipid vesicles (non-staining) also coalesce into larger droplets. Cell shape becoming irregular. In late maturation (IVb), zona radiata may thin and pull away from follicle.	
Post-Spawning	Open **post-ovulatory follicles** present in ovary indicate recent spawning. These follicles condense into a solid ball of cells within a few hours or days and are eventually reabsorbed by the ovary.	

Stage I Oocytes (Figure 6 &7)

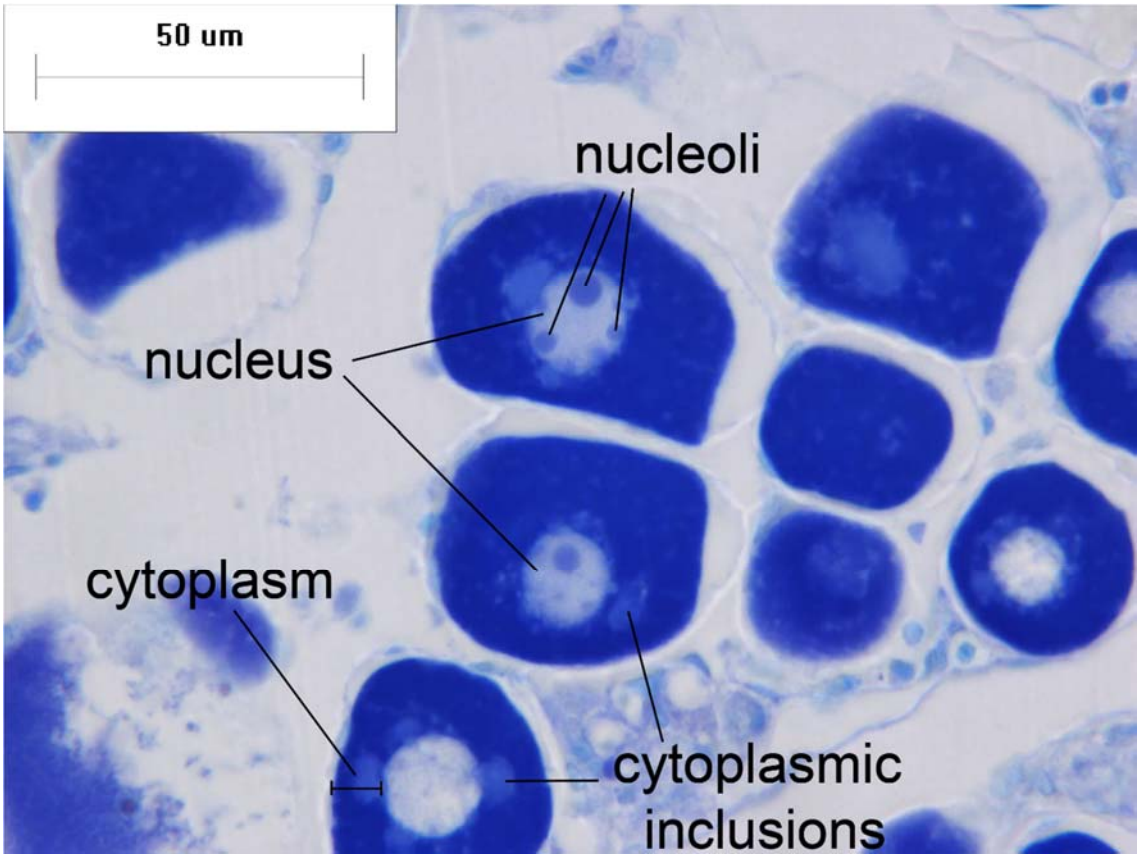


Figure 6. Stage I (primary growth) oocyte*:* **Cytoplasm stains darkly and uniformly** (but see note, below)**.** Oocyte shape is most-often angular/irregular. A circular nucleus with well-defined borders is present and prominent (> 30% of cell diameter). Nucleus may contain small nucleoli. Zona radiata is absent. Species: *Acanthurus lineatus*. Magnification: 1000X.

NOTE: In some teleosts, the majority of cytoplasm stains darkly, but lighter-staining "cytoplasmic inclusions" are present (cytoplasmic inclusions take up some stain; contrast with non-staining lipid vesicles found in Stage II oocytes, shown in Figure 8 & Figure 9).

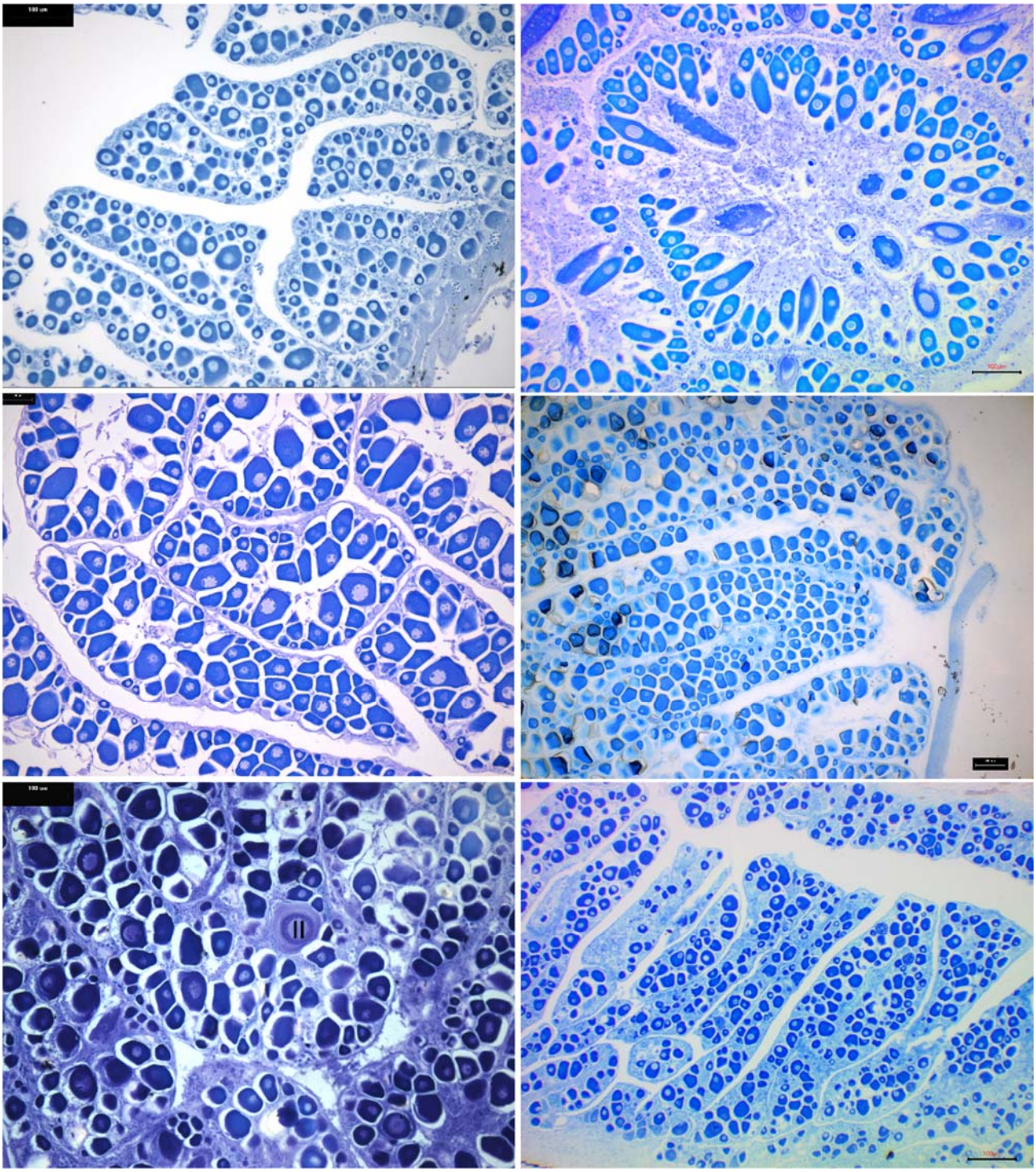


Figure 7. Examples of Stage I (primary growth) oocytes. The few Stage II (yolk vesicle) oocytes in these panels are marked accordingly. Clockwise from top left: *Acanthurus triostegus sandvicensis, Scarus oviceps, Kyphosus cinerascens, Acanthurus lineatus, Parupeneus barberinus, Scolopsis lineata.*

Stage II Oocytes (Figure 8 & Figure 9)

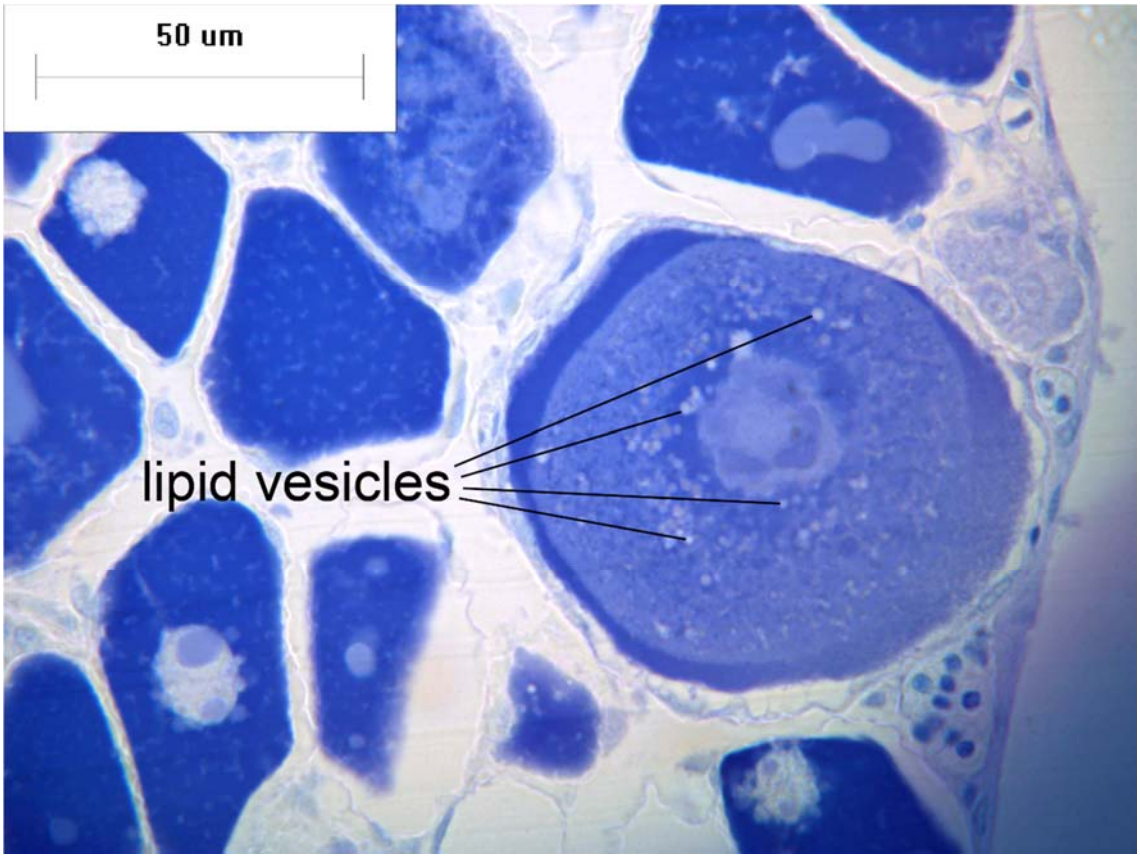


Figure 8. Stage II (yolk vesicle) oocyte: **Cytoplasm stains darkly and contains many non-staining (white) lipid vesicles.** Two- to four-times larger and outline is smoother (less angular/irregular) than Stage I oocytes. Boundary between nucleus and cytoplasm is indistinct. Mid- to late-stages have a conspicuous, lightly staining, acellular protein layer (zona radiata) surrounding the oocyte (see Stage III close-up for an example). Species: *Acanthurus nigricauda*. Magnification: 1000X.

NOTE: Early Stage II oocytes do not have a zona radiata. Also, in early Stage II oocytes, lipid vesicles tend to appear at the periphery of the cell (see example in bottom right panel of Figure 9).

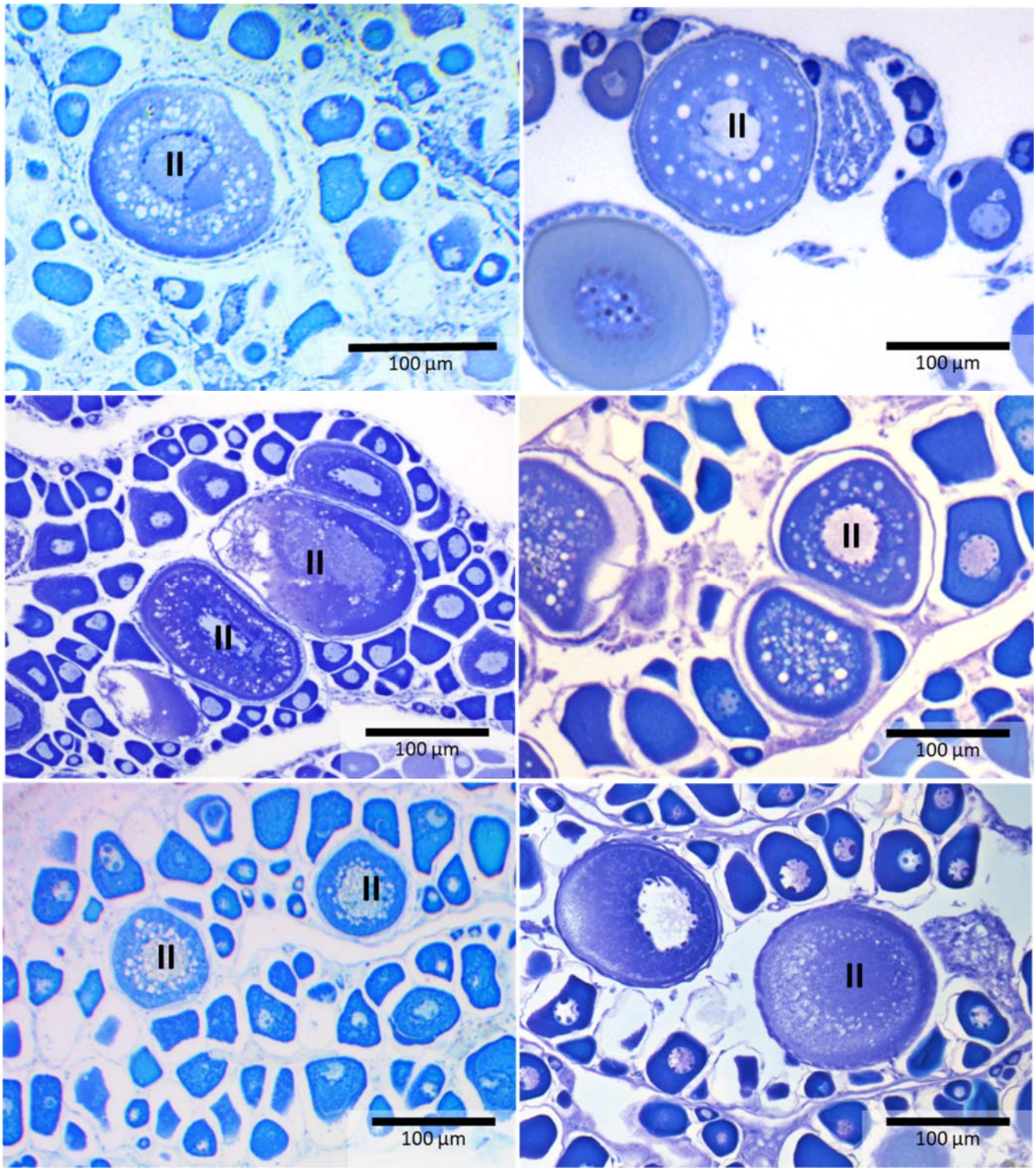
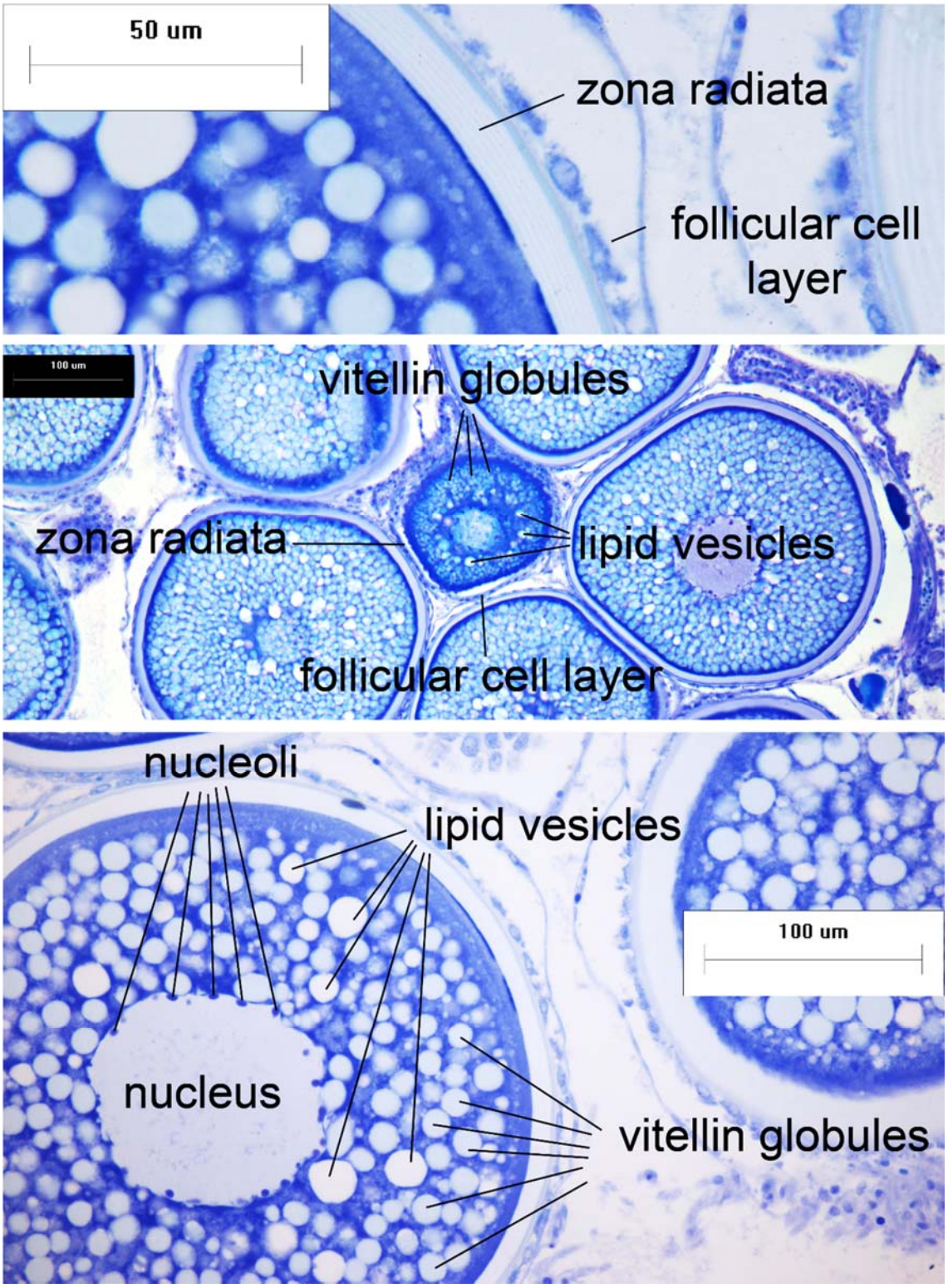


Figure 9. Examples of Stage II (yolk vesicle) oocytes. Bottom right panel shows examples of lipid vesicles first appearing at cell periphery. Not all Stage II oocytes are labelled in some panels. Clockwise from top left: *Acanthurus lineatus*, *Acanthurus triostegus sandvicensis*, *Balistapus undulatus*, *Nemipterus isacanthus*, *Ctenochaetus striatus*, and *Parupeneus multifasciatus*.

Stage III Oocytes (Figure 10 & 11)



Facing Page

Figure 10. Stage III (vitellogenic) oocyte: **Cytoplasm a mixture of non-staining lipid vesicles and stained, round, uniform, vitellin (yolk protein) globules.** Smooth edged and circular (ellipsoid in some species). Usually about two-times larger than Stage II oocytes. Nucleus usually only visible during early Stage III. Zona radiata (acellular protein layer) prominent and usually surrounded by a follicular cell layer. Top: *Lutjanus gibbus*, 1000X; middle: *Ctenochaetus striatus*, 200X; bottom: *Lutjanus gibbus*, 400X.

NOTE: Vitellin globules initially appear at the edge of the oocyte, but are seen progressively closer to the nucleus as oocyte growth continues. In some species, these globules grow in size as the oocyte progresses from early to late vitellogenesis. In the middle panel of Figure 10, a single, early Stage III oocyte is labelled (the majority of surrounding oocytes are late Stage III). A comparison of the early and late stage oocytes highlights that vitellin globules first appear at the periphery of the cell and migrate inward as the oocyte develops.

Toluidine blue (the default Jungle Histology stain) sometimes does not stain vitellin very well, making it difficult to distinguish vitellin globules from lipid vesicles. Staining with hematoxylin and eosin helps to more easily differentiate between the two elements (for more information, refer to the "Hematoxylin and Eosin Staining" section beginning on page 45).

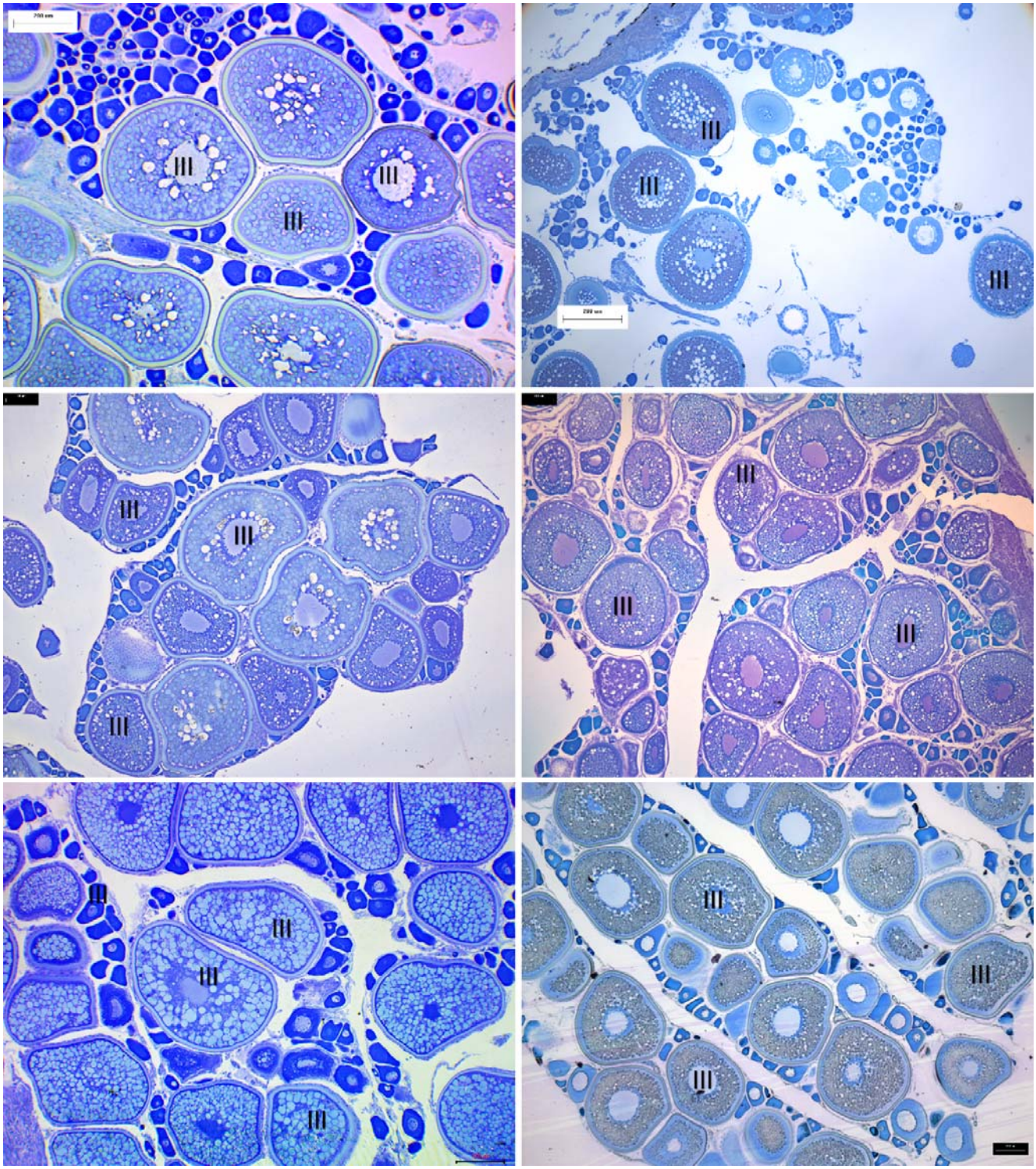


Figure 11. Examples of Stage III (vitellogenic) oocytes. Only three Stage III oocytes are labelled in each panel. Clockwise from top left: *Acanthurus nigricauda*, *Acanthurus triostegus sandvicensis*, *Balistapus undulatus*, *Ctenochaetus striatus*, *Monotaxis grandoculis*, *Scolopsis lineata*.

Stage IV Oocytes (Figure 12 & 13)

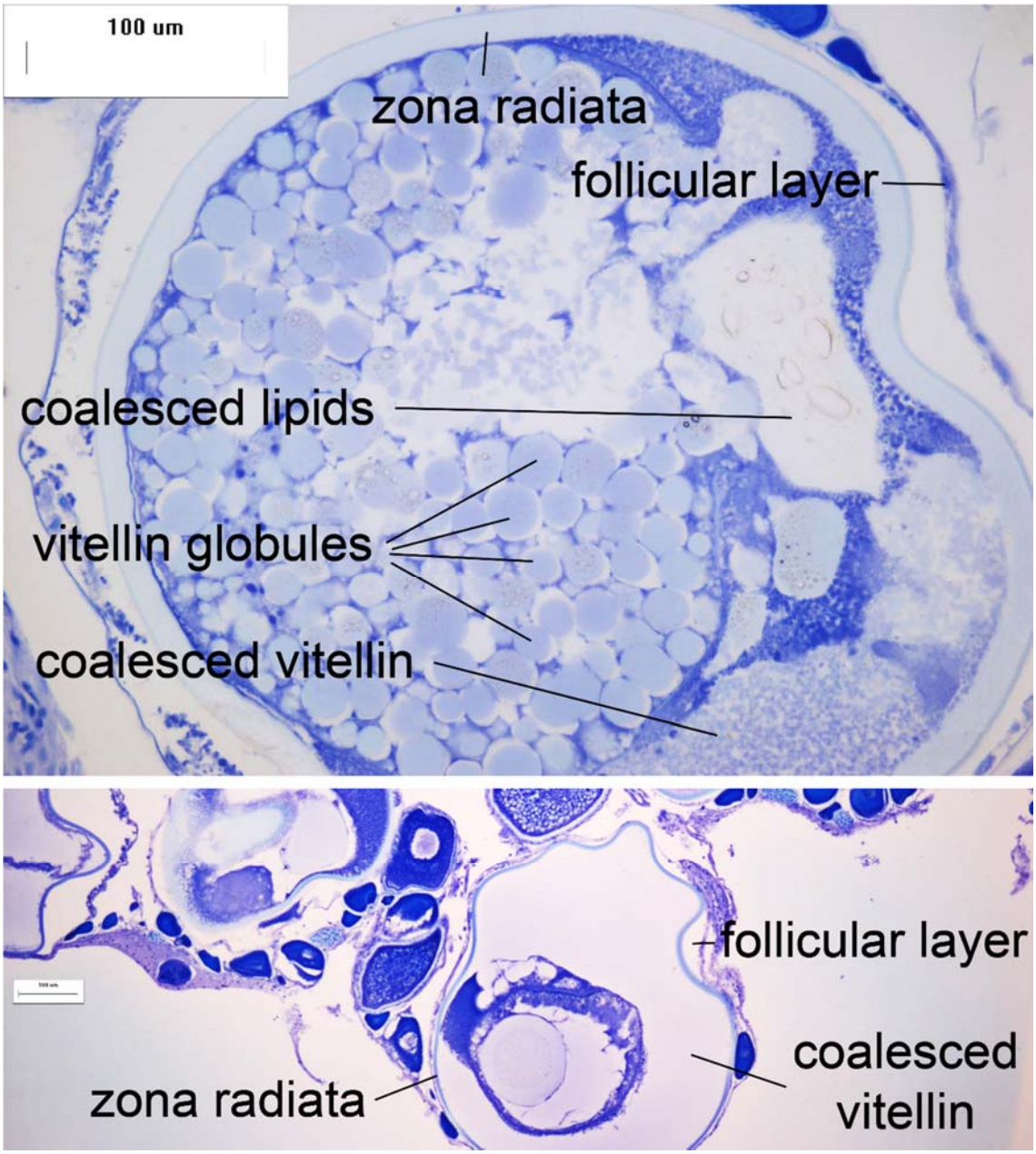


Figure 12. Stage IVa (maturation) and IVb (hydration) oocytes: **Yolk protein coalescing into larger vitellin globules (IVa) or cytoplasm a large mass of coalesced vitellin (IVb), usually staining lighter than in the previous stage.** Up to two-times larger than Stage III oocytes and less circular/ellipsoid (IVa) or very irregularly shaped (IVb). Lipid vesicles are coalescing (IVa) or may have coalesced into one or two large oil droplet(s) (IVb). Zona radiata of IVb oocytes conspicuously thinner than in Stage III. Species: *Monotaxis grandoculis*. Magnifications: 400X (top), 200X (bottom).

NOTE: The convoluted shape of Stage IVb oocytes is probably a processing artefact. We think it is caused by rapid dehydration when tissues are transferred from fixative into 50% alcohol. Distortion may be reduced if more, smaller increments are used for the alcohol dehydration series (*e.g.*, 10%, 20%, 50%, 70%, and 95%). However, the extra steps are unnecessary because the artefact does not affect stage-scoring accuracy.

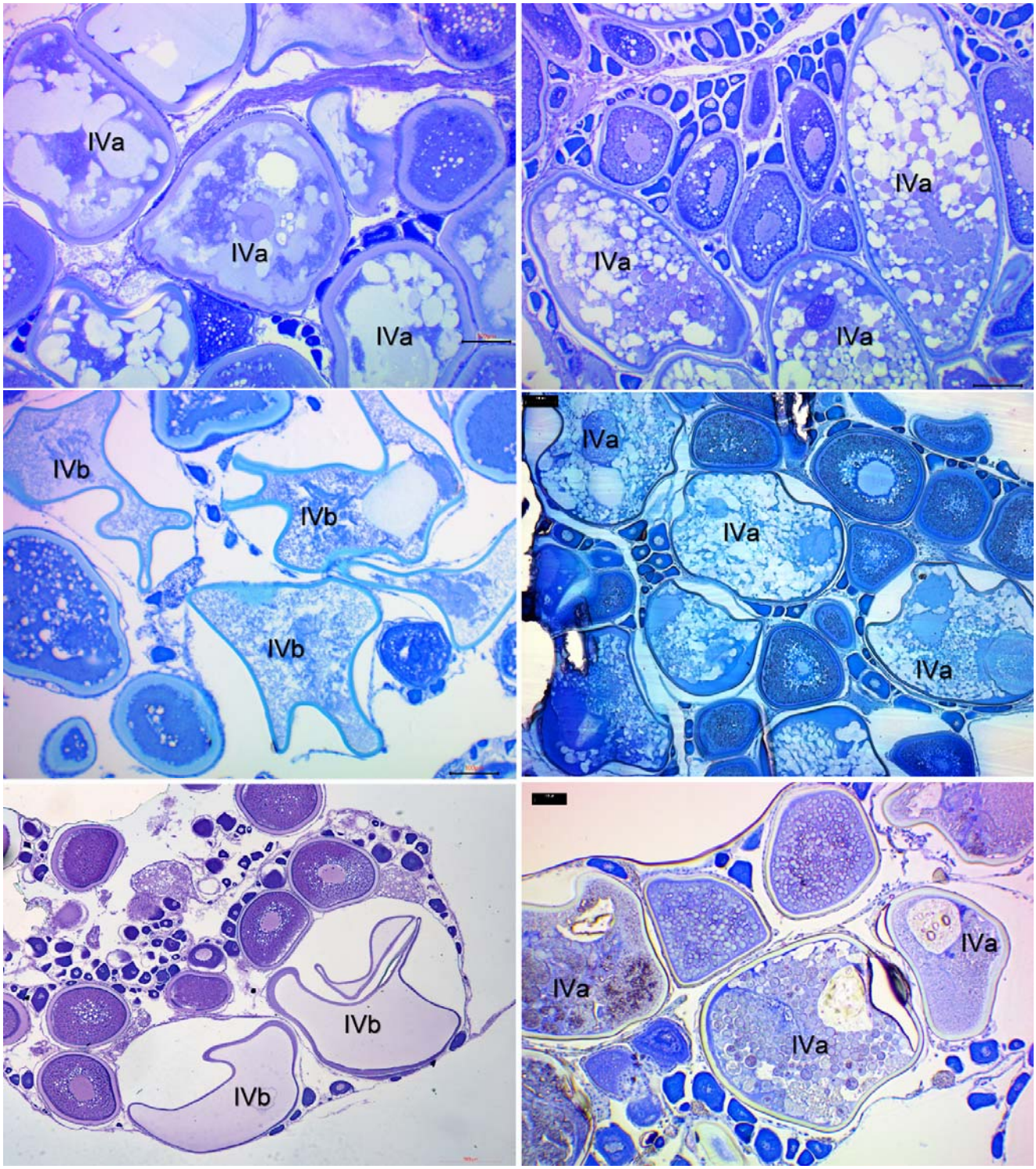


Figure 13. Examples of Stage IV (maturation and hydration) oocytes. Only three Stage IV oocytes are labelled in each panel. Clockwise from top left: *Acanthurus lineatus*, *Chlorurus japanensis*, *Caesio cuning*, *Monotaxis grandoculis*, *Lutjanus gibbus*, and *Acanthurus nigricans*.

Post-Ovulatory Follicles (Figure 14)

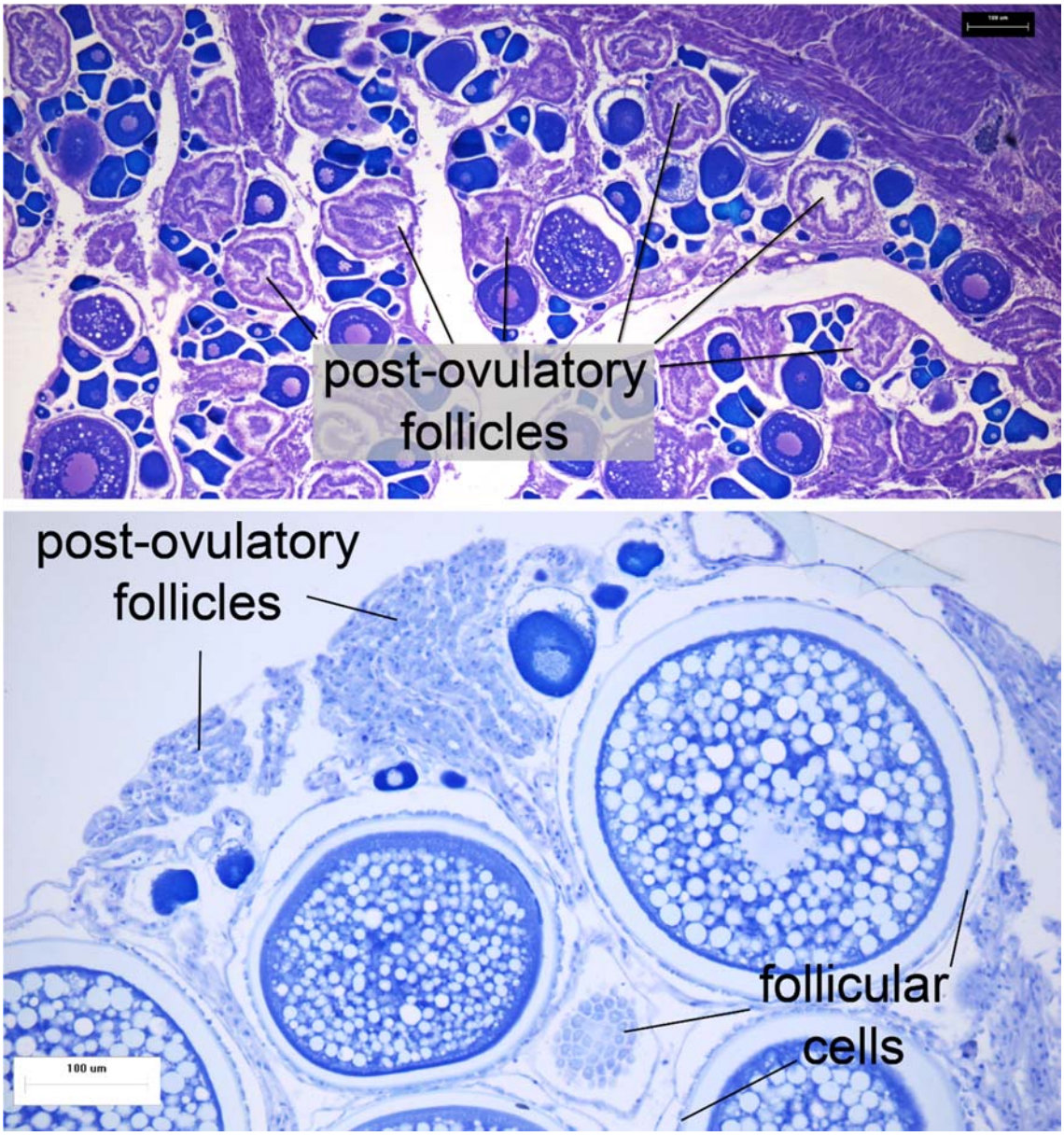


Figure 14. Post-ovulatory follicle (POF): **A collapsed band of follicular cells** remaining within the ovary after the ovulation of the previously enclosed oocyte. New POFs are usually one-half the diameter of Stage III oocytes and contain a conspicuous lumen (see top panel). Top: *Balistapus undulatus*, 100X; bottom: *Lutjanus gibbus*, 200X.

NOTE: Post-ovulatory follicles remain in the ovary after ovulation, but degenerate quickly. Their presence indicates recent spawning. Individuals with POFs should be considered mature.

MALES

Testicular Anatomy

Testes are the gonads of males. In most fishes, the testes are bi-lobed, although one lobe may be much larger than the other. When sectioned transversely, they usually appear triangular to bean-shaped. The testis is surrounded by a thin gonad wall composed primarily of fibrous connective tissue. Internally, the testis is divided into numerous **lobules** (Figure 15). These are blind-ended tubules consisting of an outer layer of spermatogenic (sperm-producing) tissue surrounding a lobule lumen (note that the lumen may not be visible in all individuals). Mature sperm are shed into the lumen and empty into a **sperm duct**, which is most-often located in the medial part of the gonad. When sampling a testis, it is important to include the sperm duct; in mature individuals, it will likely contain sperm.

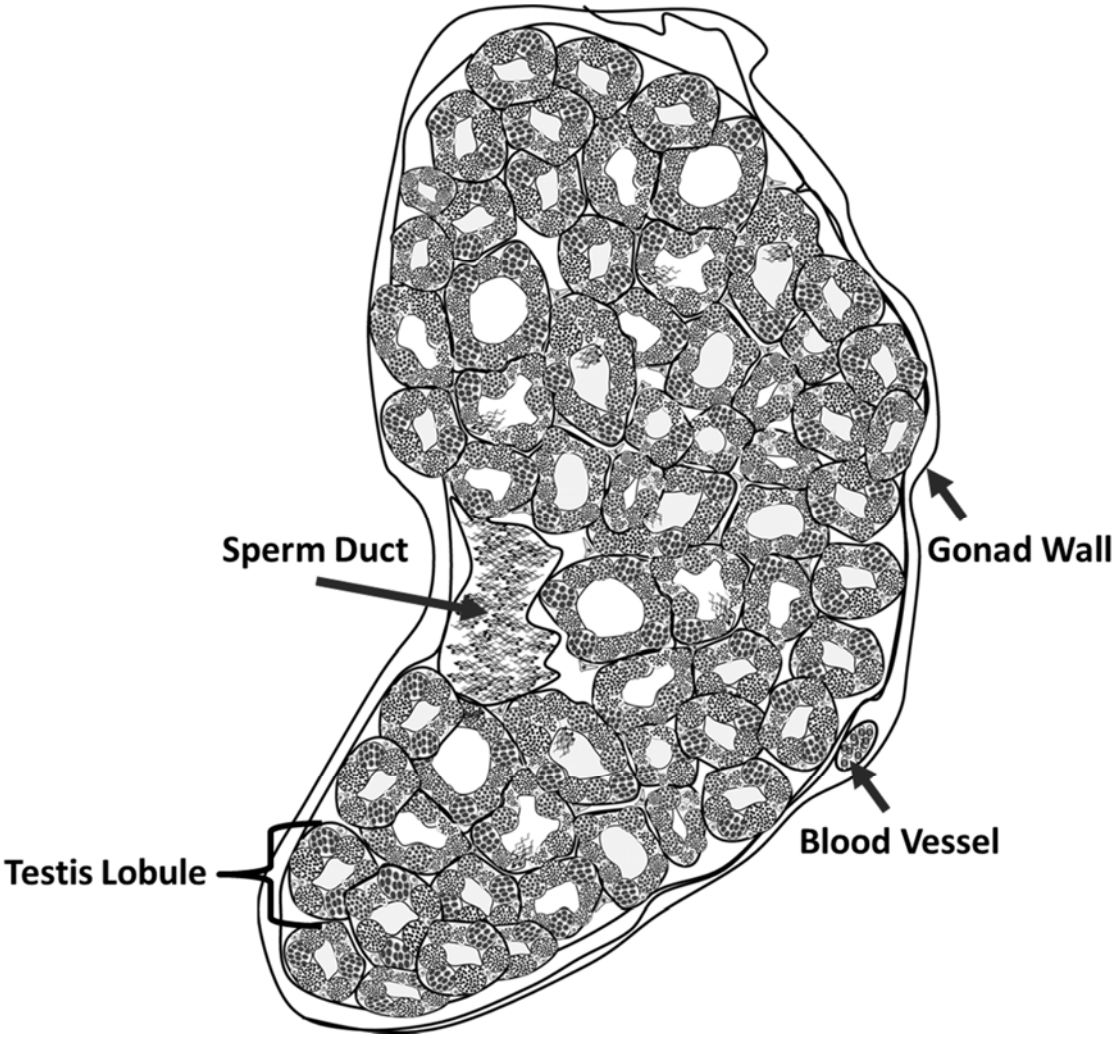


Figure 15. Idealized anatomy of a coral-reef fish testis.

Sperm Development

Spermatozoa are the gametes of mature male fishes. They consist of a head containing the male's genetic contribution to the future embryo (*i.e.*, a single set of chromosomes), and a flagellum (tail) that propels the sperm towards the egg for fertilization.

All sperm are ultimately derived from **spermatogonia** (Figure 16), immature reproductive cells located in the testis. The process by which a spermatogonium becomes a mature sperm (**spermatogenesis**) is complex, involving cell division and hormonal signals from the brain and **leydig cells** found between the testis lobules. Leydig cells produce testosterone, which helps to stimulate spermatogenesis and is responsible for male secondary sex characteristics (*e.g.*, courtship behaviour and coloration). During spermatogenesis, dividing spermatogonia become encapsulated by **Sertoli cells** to form **spermatocysts** (capsules of germ cells in the same stage of development). As the cells within each spermatocyst divide, their nuclei become progressively smaller. Thus, nucleus size can be used to distinguish primary **spermatocytes**, secondary spermatocytes, and **spermatids**. The latter differentiate (without dividing) into spermatozoa. Thus the nuclei of spermatids (essentially spermatozoa without tails) and spermatozoa are approximately the same size. These cells can be distinguished by the presence or absence of flagella. Eventually, mature spermatozoa (motile – or tailed – sperm) erupt from the cyst in a process known as **spermiation.** Once released, the sperm enter the lobule lumen and are transported out of the body via sperm ducts.

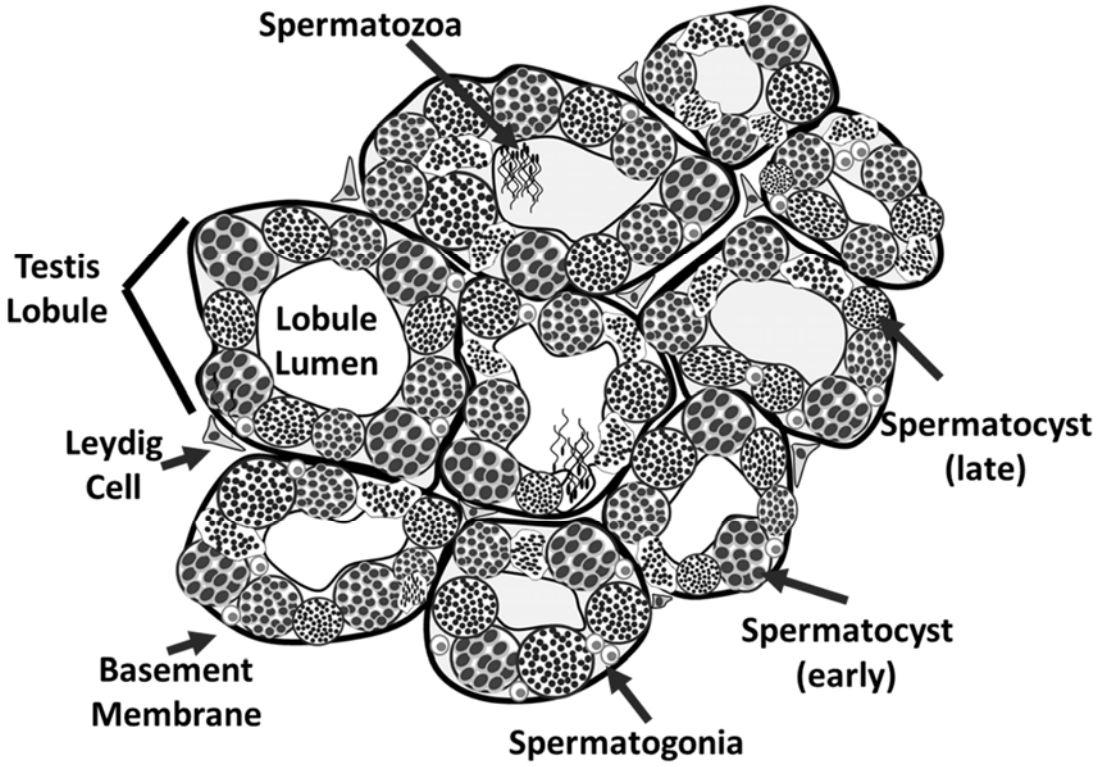


Figure 16. Microscopic structure of a mature coral-reef fish testis.

Male Germ-Cell Classification

Unlike for ovaries, it is usually unnecessary to classify each type of germ cell in fish testes. This is because testis histology is rarely used to describe seasonal trends in reproductive effort for male coral-reef fishes. Although testes often show histological signs of regression (*i.e.*, fewer late-stage spermatocysts and fewer spermatozoa) during "off seasons", in mature males, sperm are often present even during non-spawning periods (sperm are "cheap" to produce, so it makes sense to have some around all the time).

A simple scan of testicular sections for the presence or absence of tailed spermatozoa will indicate whether a male is mature or immature. Testes that contain tailed sperm are considered mature; those that do not are considered immature. Begin a scan by observing the medial sperm duct(s) and lobule lumina. During the spawning season, mature males will typically have copious quantities of sperm in both the duct and lumina. Be aware, however, that other materials (*i.e.*, cellular debris and testicular secretions) may also be present in the duct. Consequently, it is essential to view the section at high magnification (400x or 1000x) and judiciously use the microscope's fine adjustment to resolve the diagnostic "tails" (flagella) that distinguish sperm from other cells. Flagella do not stain well with toluidine blue, so it may be helpful to use a counterstain such as eosin (see subsection on hematoxylin and eosin staining) to better resolve the tails. Alternatively, placing a drop of water and coverslip over the section sometimes imparts a temporary light-violet appearance to the flagella, making them easier to see (see subsection on wet cover-slipping). If sperm are not found in the "open spaces" (lumina and duct) of the testis, focus on the spermatocysts making up the wall of the lobule. Remember, the most advanced spermatocysts will contain small cells with pinpoint nuclei. For these, simply determine whether cells have tails (spermatozoa) or not (spermatids).

Finally, a word about sectioning thickness. Although oocytes can be observed and classified over a wide range of section thicknesses, testes are most reliably classified from particularly thin sections. Thus, we advise that extra care be taken during the sectioning phase to ensure that some thin sections are obtained. Prior to staining, the best sections resemble tiny, translucent strips of cellophane and may have a "rainbow-like" sheen. When floated on water droplets (i.e., mounted on a slide), the sections quickly flatten and become completely transparent. Once dried and stained, these thin sections will yield high-quality preparations with a minimum of cellular overlap (*i.e.*, flagella will be easier to see).

Immature Testes (Figure 17 & Figure 18)

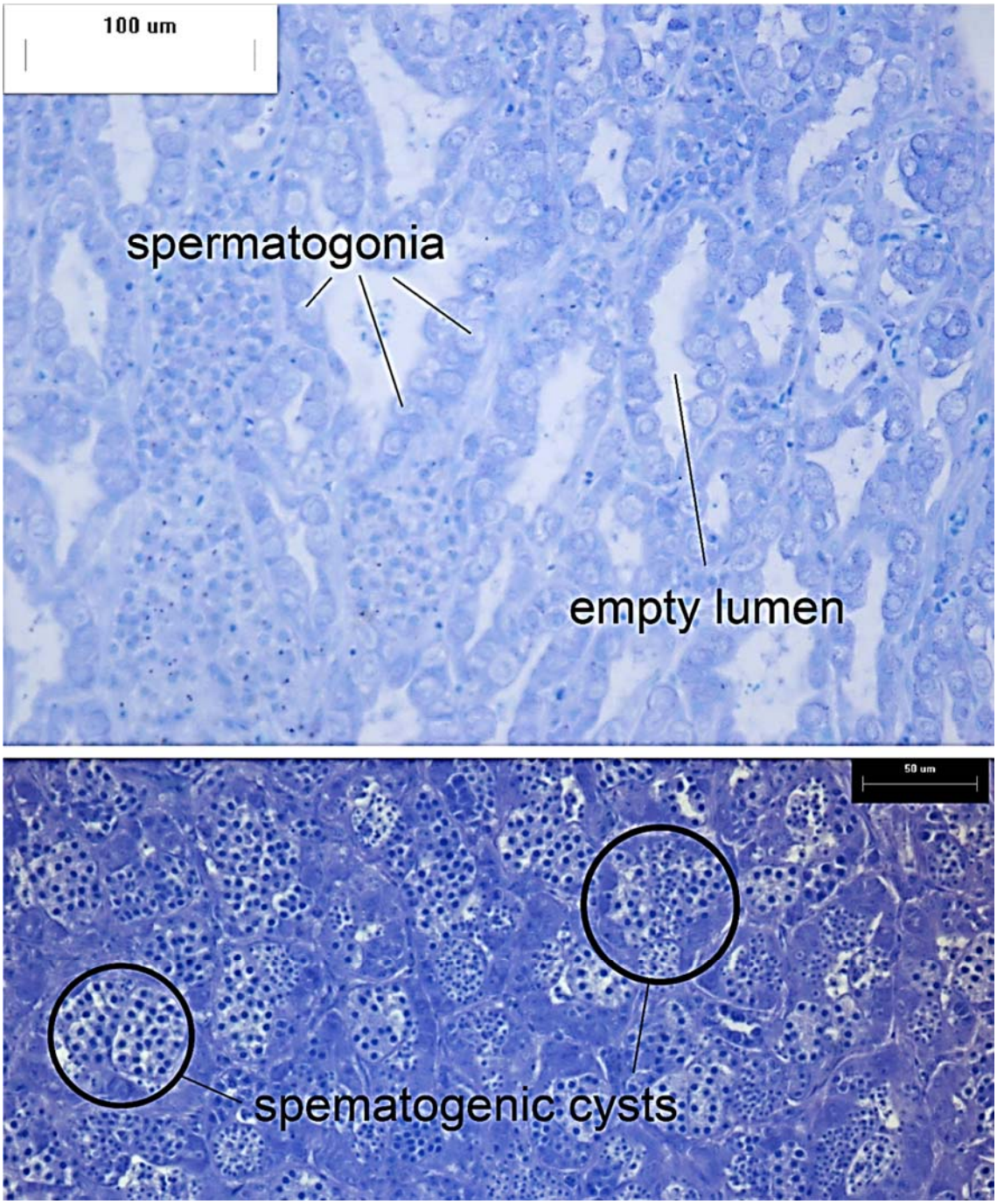


Figure 17. Immature testis: **Absence of spermatozoa (motile male gametes as evidenced by the presence of flagella or "tails").** *Monotaxis grandoculis*, 400X (top); *Nemipterus isacanthus*, 400X (bottom).

Note: Immature testes may contain a variety of structures and cell types, including: spermatogonia which are early-stage germ cells (top panel), and spermatogenic cysts with cells in various stages of differentiation (bottom panel). Lumina, if present, will not contain spermatozoa.

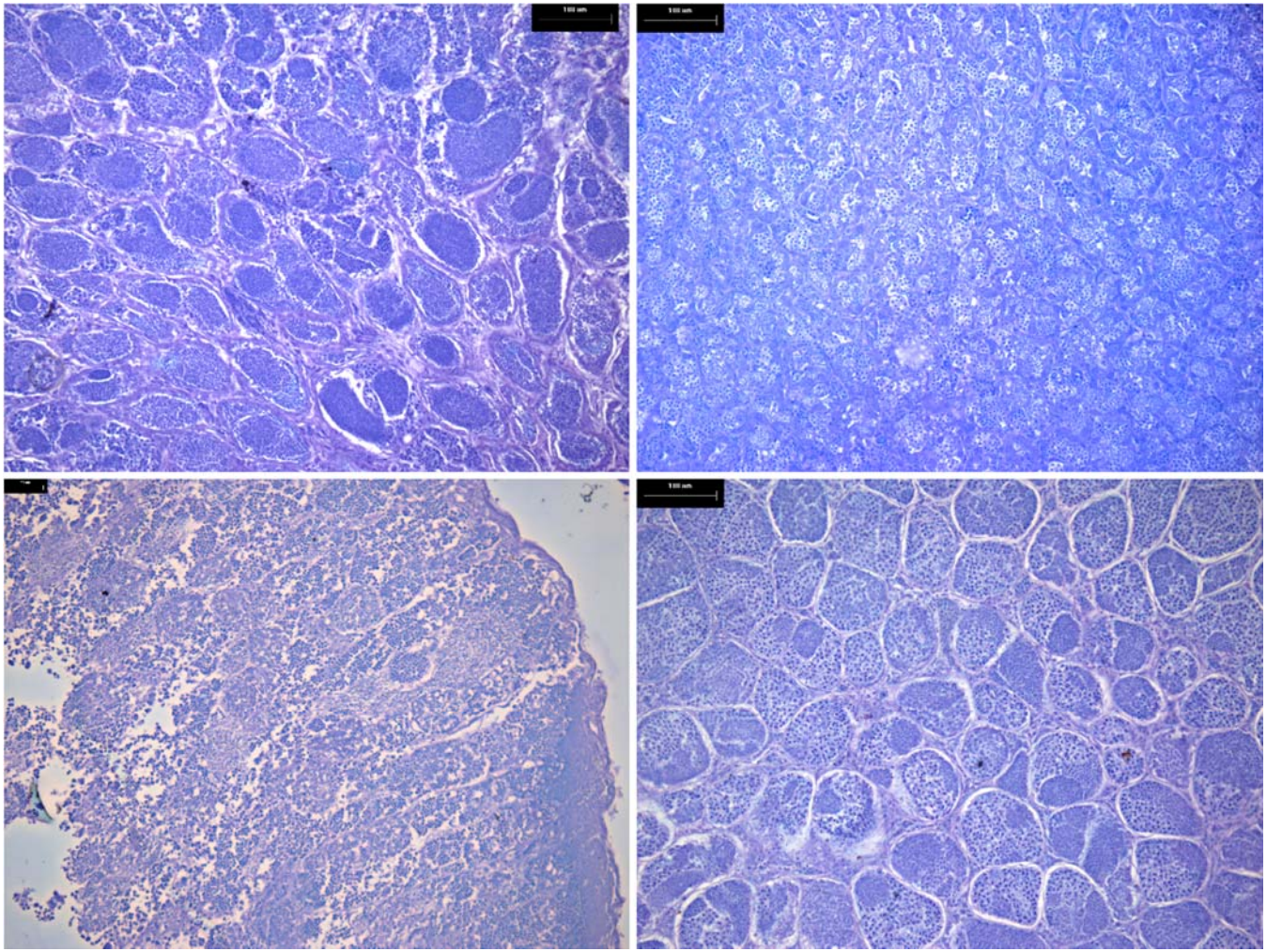


Figure 18. Examples of immature testes. Clockwise from top left: *Balistapus undulatus*, *Nemipterus isacanthus*, *Parupeneus barberinus*, *Scolopsis lineata*.

Note: The lobules in the bottom left panel have been longitudinally sectioned. The other panels show transverse (or cross) sections.

Mature Testes (Figure 19 & Figure 20)

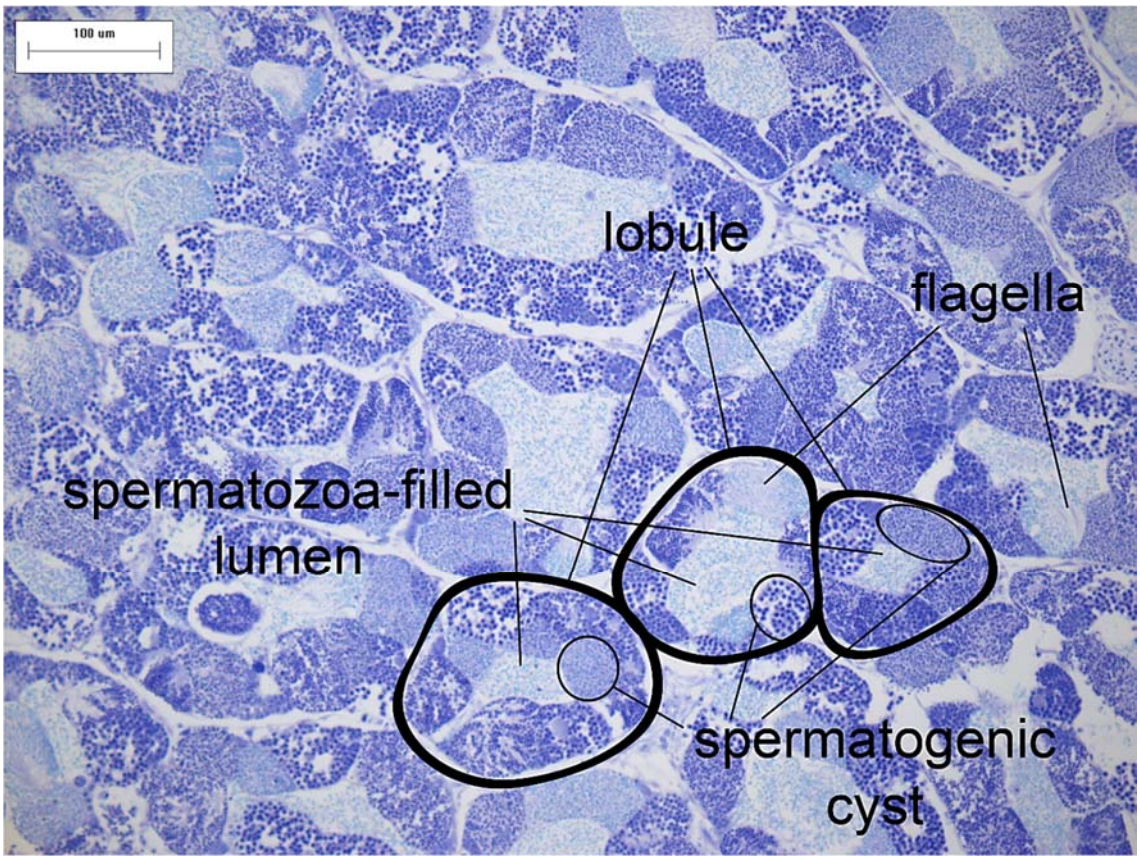


Figure 19. Mature testis: **Presence of spermatozoa (as evidenced by the presence of flagella or "tails").** Species: *Acanthurus lineatus*; Magnification: 200X.

Note: Mature testes may contain some or all of the structures and cell types seen in immature testes, the presence of flagellated spermatozoa is the criterion for maturity.

Figure 19 nicely illustrates the testis structure of most coral-reef fishes. Lobules (or blind-ended ducts) begin near the periphery of the testis, run toward the center of the testis, and open into an efferent duct (which ultimately leads to the exterior of the fish). Each lobule, shown here in transverse sections, is lined by spermatogenic cysts. Each cyst contains clonal germ cells in the same stage of spermatogenesis. Note that late-stage spermatocysts contain cells with smaller nuclei compared to the cells in early-stage spermatocysts. Spermatozoa eventually erupt from the cysts and enter the central lumen of the lobule.

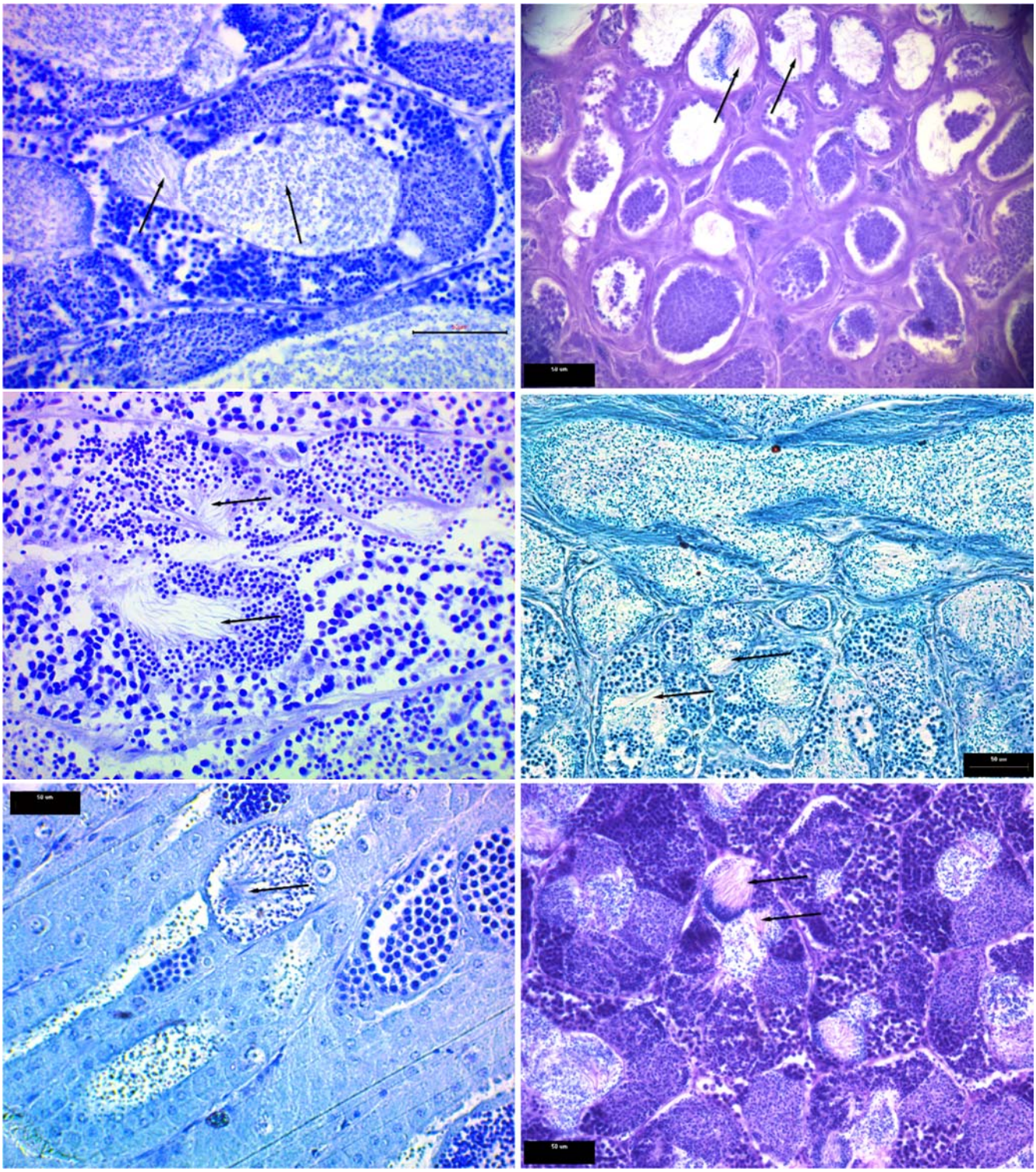


Figure 20. Examples of mature testes. Arrows indicate examples of flagella. Clockwise from top left: *Acanthurus lineatus*, *Balistapus undulatus*, *Caesio cuning*, *Ctenochaetus striatus*, *Lutjanus gibbus*, and *Scarus oviceps*.

UNDIFFERENTIATED GONADS (Figure 21)

Estimating the size at which 50% of individuals of that size are mature (L_{50}) requires sectioning gonads from many immature and mature individuals. Some of the smallest immature individuals may have undifferentiated gonads (*i.e.*, not identifiable as either sex). In most cases, these undifferentiated gonads will consist primarily of stromal (connective) tissue intermixed with a few gonial cells. Distinguishing these gonads from non-gonadal tissue can be quite difficult. Microscopically, they often can be identified as gonads only by their position relative to other structures present in the section (*e.g.*, kidney, gut, and swim bladder). Macroscopically, undifferentiated (and immature) gonads are often not visible to the naked eye. To increase the likelihood of obtaining reproductive data from the smallest individuals, we recommend a liberal approach to sampling tissues for histological analysis (*i.e.*, do not attempt to excise only gonad, rather include tissues surrounding the area where the gonad is expected to be located).

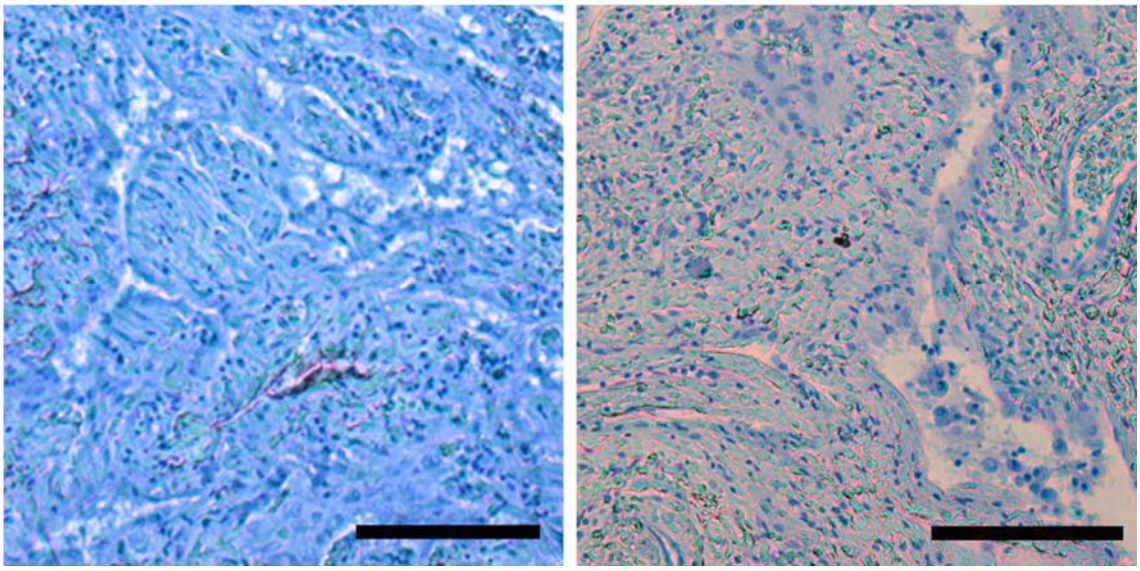


Figure 21. Undifferentiated gonads. These gonads are found only in the smallest individuals and consist primarily of gonial cells scattered within stromal tissue. Gonial cells have not differentiated into oogenic or spermatogenic lineages (i.e., sex cannot be determined). Left: *Lutjanus semicinctus*, 100X; right: *Lutjanus timorensis*, 200X. Scale bars = 100 µm.

OTHER FEATURES

Ovarian Lumen (Figure 22)

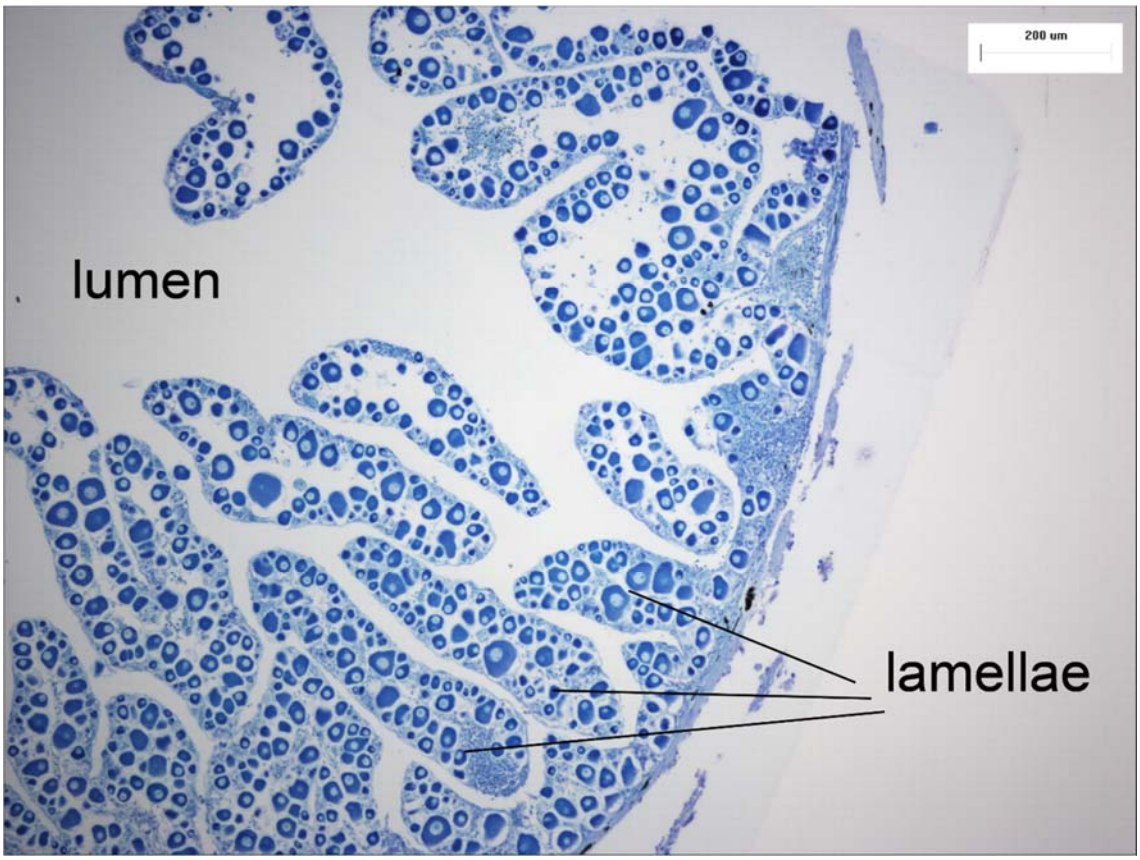


Figure 22. Ovarian lumen. Oocyte-containing lamellae project from the ovarian wall and into a membrane-lined cavity (lumen). Species: *Acanthurus triostegus sandvicensis*; Magnification: 100X.

Note: In mature females, ova erupt from the lamellae (*i.e.*, are ovulated) to enter the lumen and ultimately exit the body via the urogenital pore.

Testes of "normal" (i.e., non-sex changing) males do not have a central lumen. Thus, the presence of a membrane-lined lumen in testes may be evidence of sex change.

Atretic Oocyte (Figure 23)

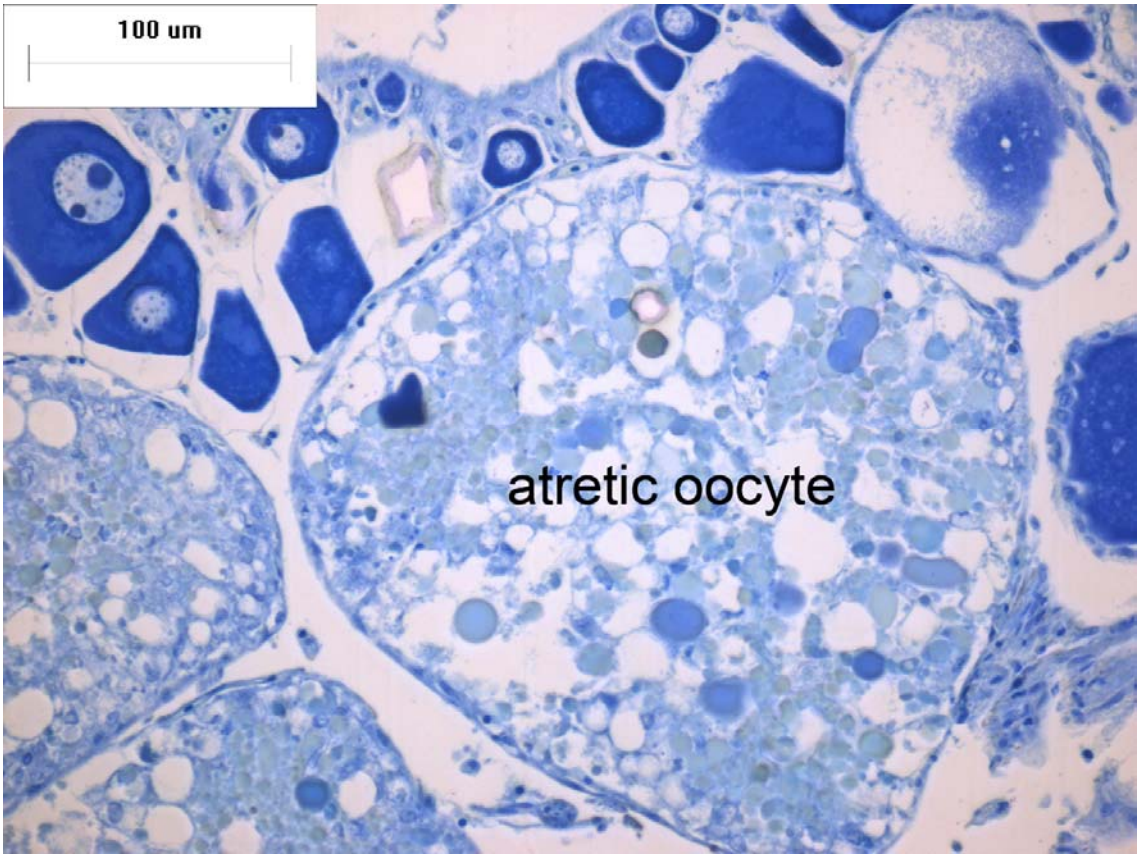


Figure 23. Atretic oocyte. Characterized by the disintegration of the nucleus, vitellin globules, and zona radiata. Species: *Lutjanus gibbus*; Magnification: 400X.

Note: Atresia is the breakdown of oocytes and can occur in any stage of the ovarian cycle. However, atretic oocytes are not always present. They are most common in the post-spawning period.

An improperly preserved (rotting) ovary may appear to contain atretic oocytes; however in these cases *all* oocyte stages will show a similar degree of degradation. In contrast, Stage I and II oocytes do not typically undergo atresia in a "resting" ovary (the period immediately after spawning and before the next batch of oocytes begins differentiating). Also, in a rotting ovary, lamellae will show signs of degradation and there may be lots of debris in the spaces between oocytes.

Given all of the above, in a properly preserved ovary, atretic oocytes may be considered evidence of maturity.

Finally, follicular atresia may occur during sex change in protogynous species.

Melano-Macrophage Centers (Figure 24)

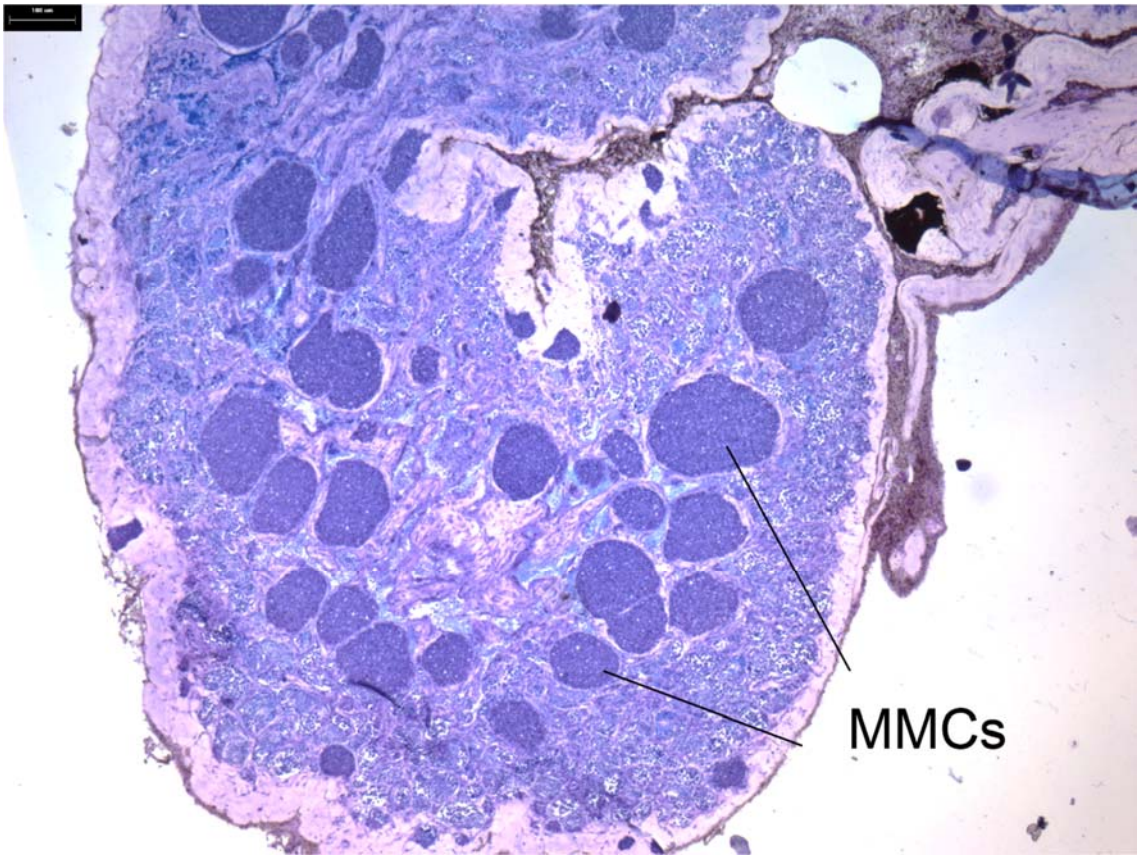


Figure 24. Melano-macrophage centers (MMCs). These are distinctive groupings of pigment-containing cells. Species: *Ctenochaetus striatus*; Magnification: 100X.

Note: MMCs are occasionally present in many organs (they are not restricted to gonads). MMCs stain quite variably with toluidine blue, sometimes they will be yellowish and other times they will stain darkly as in Figure 24. The production of MMCs, or yellow-brown bodies, from vitellogenic oocytes is a well-documented process. When found in ovaries, MMCs may be evidence of maturity. When found in testes, MMCs are weak evidence of sex change. However, MMCs may have other causes (*e.g.*, infection and subsequent inflammatory processes, or environmental stress). Thus, MMCs, alone, do not provide enough evidence to diagnose sex change or assign reproductive maturity.

OVOTESTES and Transitional Gonads (Figure 25)

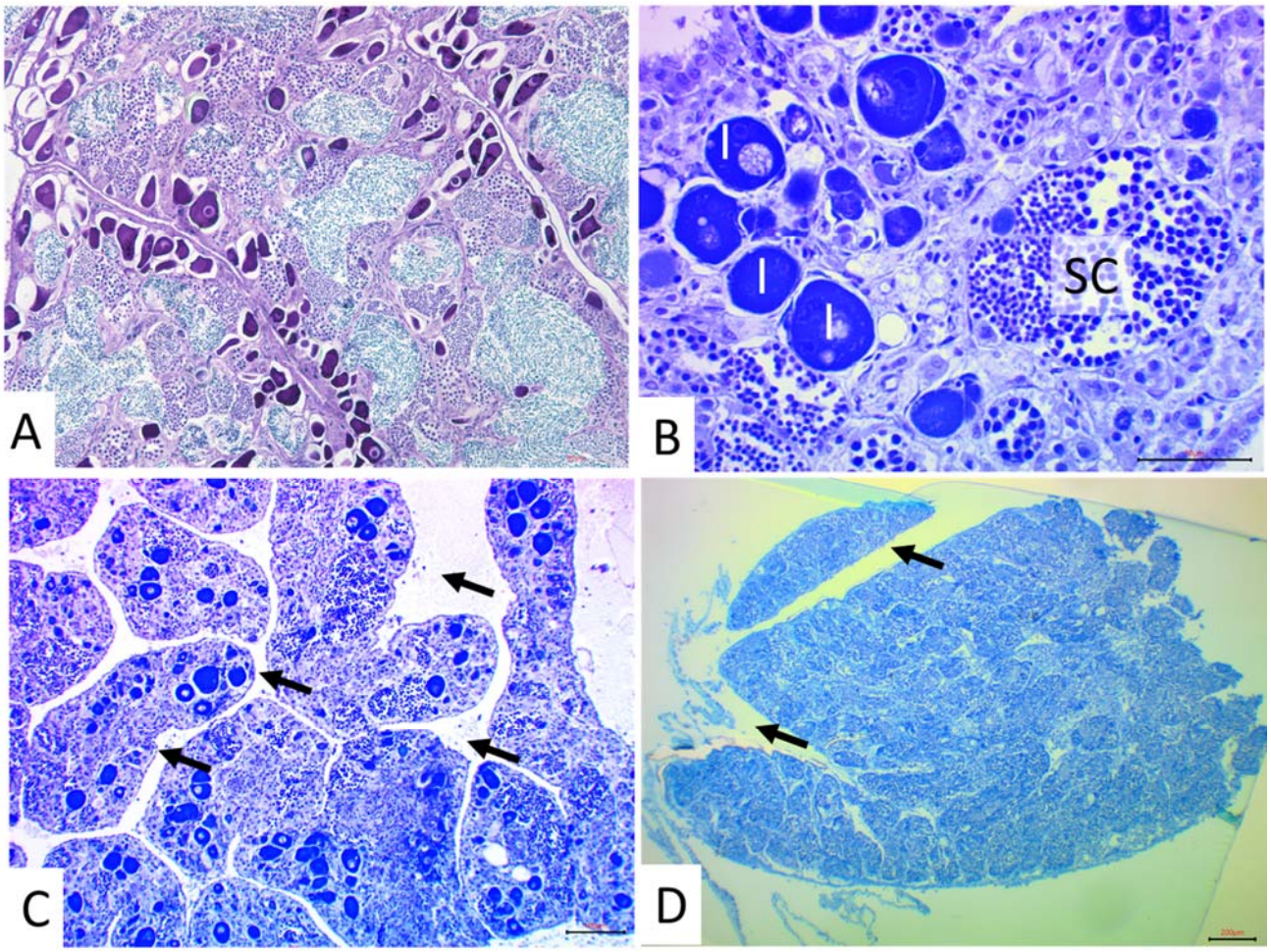


Figure 25. Transitional gonads show evidence of sex change. Included in this category are ovotestes, which contain a mixture of spermatogenic tissue and ovarian tissue (A-C). In protogynous species (female to male sex changers), these ovotestes may contain remnants of the former ovarian lumen (arrows). When present in testes of mature males (D), this lumen is suggestive of previous ovarian/female function. Species: *Plectropomus oligacanthus* (A-200x) and *Scarus oviceps* (B-D) Magnification 400x (B) and 100x (C&D). I = stage I oocytes. SC = spermatocysts.

Note: When relatively frequent (*e.g.*, ≥ 3% of specimens), ovotestes may be evidence that a species changes sex. Melano-macrophage centers (MMCs) in testes may suggest previous female function.

A variety of environmental challenges can cause Stage I or II oocytes to appear in testes. In addition, many species have bisexual gonads as juveniles, and one type of tissue degrades before the individual matures. In either situation, an individual only ever *functions* as one sex (*i.e.*, it does not *change* sex). Investigators that find ovotestes should carefully read Sadovy & Shapiro (1987) before concluding that a species changes sex. In true sex change (*i.e.*, sequential hermaphroditism), an individual initially *matures* as one sex then changes to the other. To search for definitive evidence of sequential hermaphroditism, all ovotestes from a single species should be examined to determine whether there is a clear progression from one sex to another.

NON-GONADAL TISSUES AND STRUCTURES

On a gross (macroscopic) level, some structures may be mistaken for gonads when processing a whole fish specimen. For example, fat bodies (adipose tissue) may be unintentionally removed because they resemble mature testes. Gut may be removed because it looks like an immature gonad from either sex. When these mistakes are made, prepared slides will only contain only non-gonadal tissue.

Other structures may be attached to small gonads when they are removed from a specimen. Those structures may be seen (and cause confusion) on prepared slides (Figure 26).

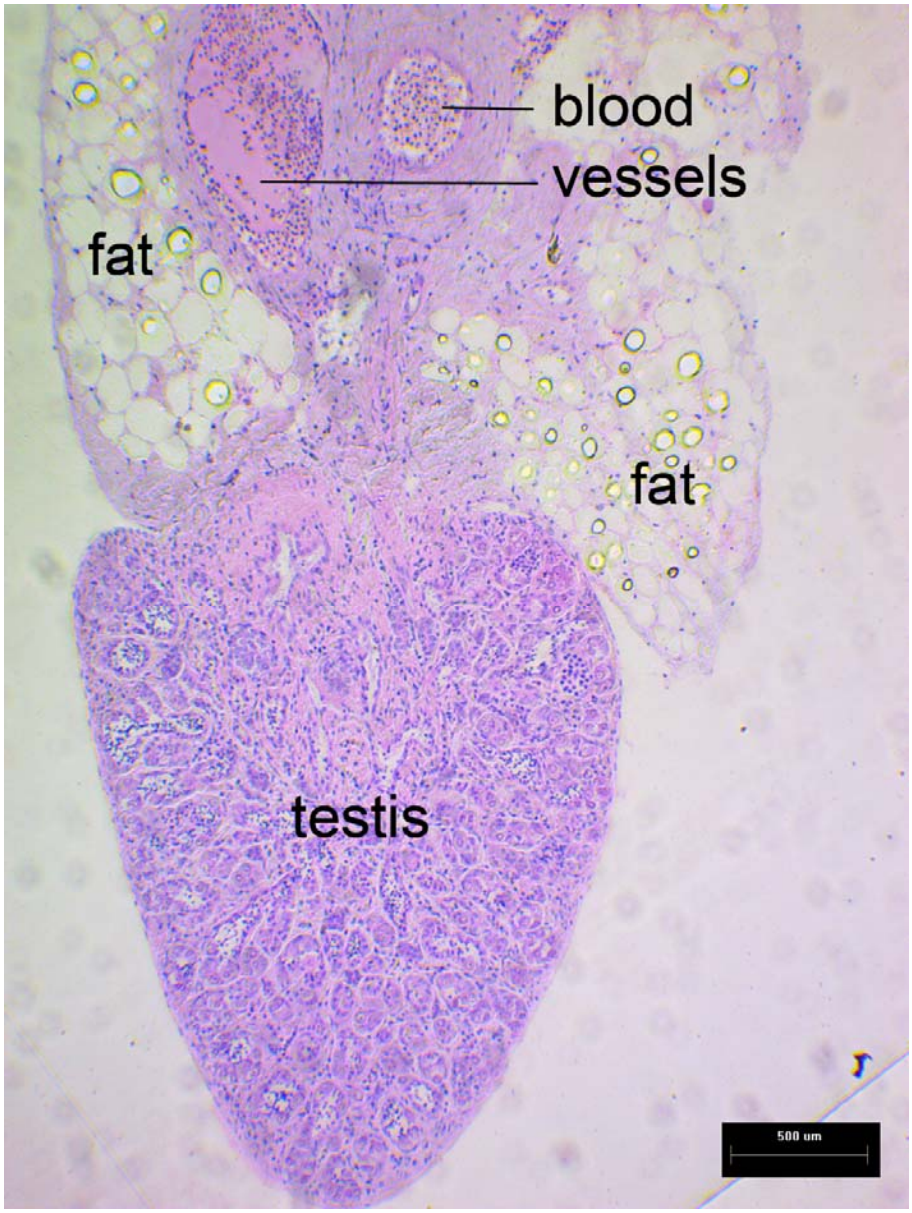


Figure 26. A tissue section including non-gonadal tissues and structures. *Lutjanus gibbus*, 40X.

Note: These sections have been stained with hematoxylin and eosin, rather than toluidine blue (used for all previous examples). A protocol for hematoxylin and eosin staining, and examples of the results, are presented in a subsequent section.

Fat (Figure 27)

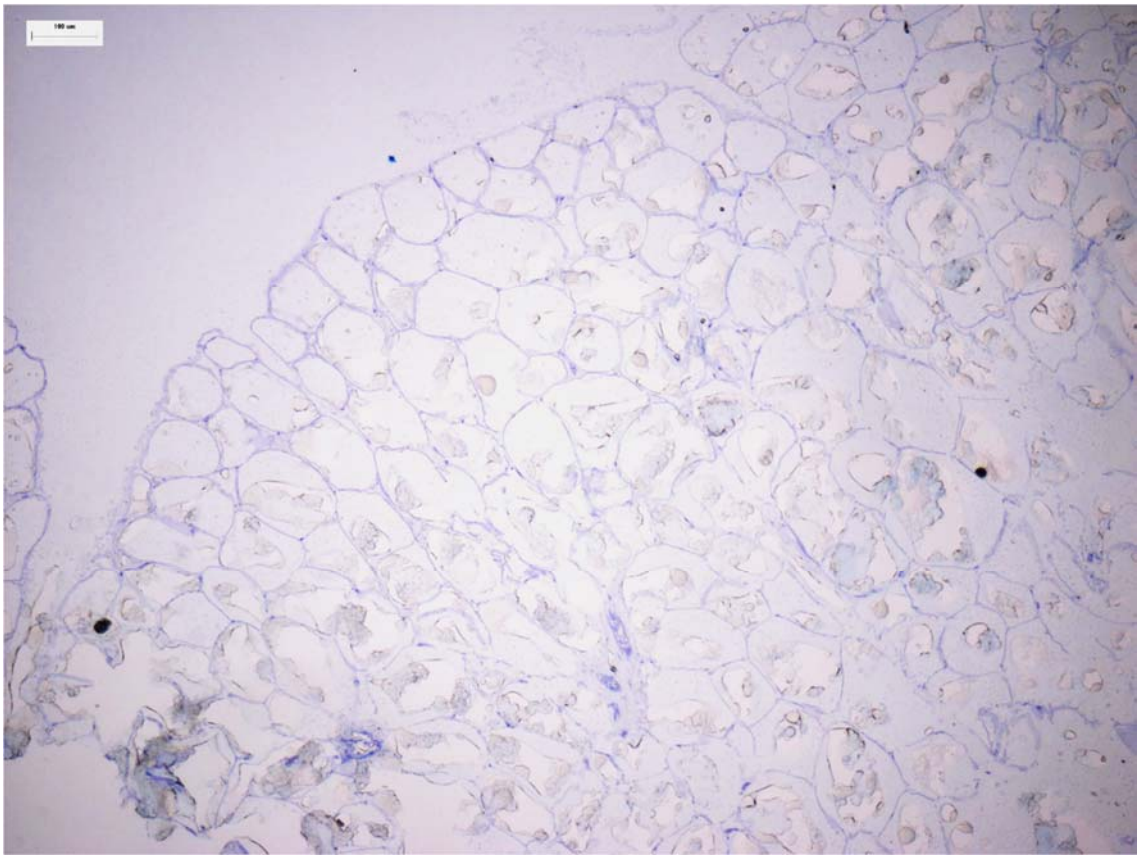


Figure 27. Fat (adipose tissue). Fat does not take up stain very well. Nor does it infiltrate or embed very well. Because of these two characteristics, prepared sections that contain fat are often lightly colored and torn. Species: *Acanthurus lineatus*; Magnification 100X.

Gut (Figure 28)

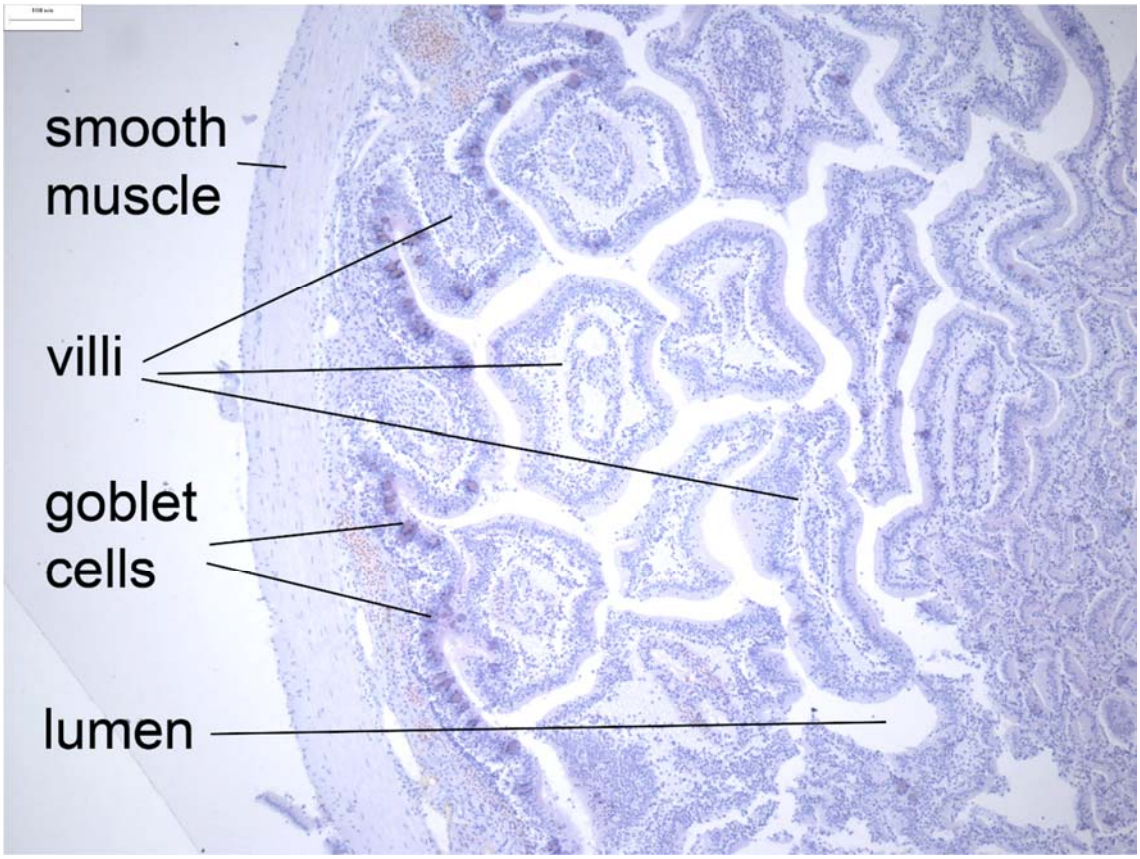


Figure 28. Gut. At first glance, gut may be mistaken for an ovary; a lumen is present, villi in longitudinal section resemble ovarian lamellae, and villi in transverse section (with their irregular shape) resemble Stage IV oocytes. However, zona radiata are absent and the villus contains many dark-staining dots, each being the nucleus of an individual cell (oocytes are a single cell and have only one nucleus). Species: *Acanthurus nigricauda*; Magnification 100X.

Blood (Figure 29)

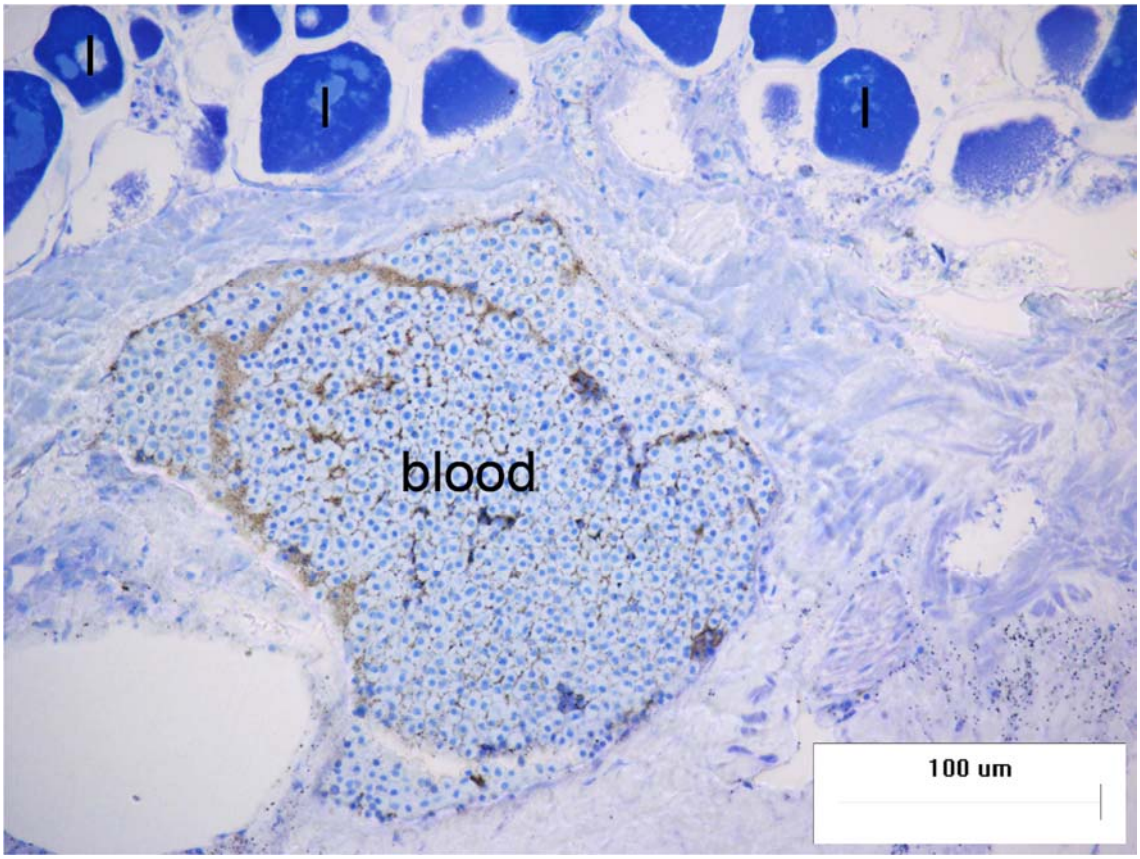


Figure 29. Blood. Blood cells of fish are nucleated and may superficially resemble spermatocytes. Compare with Figure 17 & Figure 18 (immature testes) and note that the diameter of blood vessels is larger than that of spermatogenic cysts. In addition, unlike spermatogenic cysts, blood vessels are comparatively rare and do not occur in closely packed groups. Blood cells are oval in shape and have large amounts of cytoplasm whereas spermatogenic cells tend to be circular and contain mainly nucleus.

STAINING ADVICE

The Jungle Histology method of staining with toluidine blue - although easy, fast, and inexpensive - can make it difficult to distinguish between mature and immature gametes of both sexes. Because almost *everything* in an oocyte is stained some shade of blue, it can be challenging to determine whether vitellin granules are present (*i.e.*, to diagnose maturity in females on the cusp of vitellogenesis). Also, toluidine blue has a low affinity for the tails of spermatozoa, and because spermatids and the heads of spermatozoa are nearly the same size (spermatids do not divide before maturing into spermatozoa), it can be difficult to distinguish immature and mature males.

Below are two methods to help increase the contrast between immature and mature gametes on slides prepared with Jungle Histology methods.

Wet Cover-Slipping

Wet cover-slipping is a simple trick that *sometimes* helps to resolve key structures by imparting a temporary color change in gonad sections stained with toluidine blue. Adding a drop of water and a cover slip increases the degree of contrast and hides knife-nicks or other imperfections. Often, wet cover-slipping will result in lavender-tinted vitellin granules, making them easier to resolve against the dark blue background of early stage III oocytes (Figure 30). In addition, when the technique is used on testis sections, it will usually impart a bright purple color to spermatozoa and spermatids while earlier-stage spermatogenic cells remain blue (Figure 31).

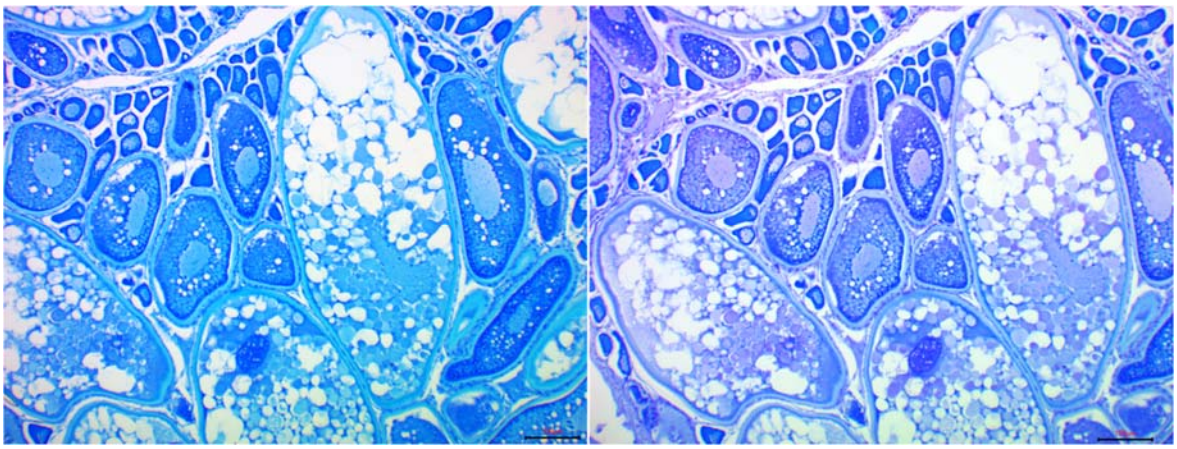


Figure 30. Effects of wet cover-slipping on an ovarian section stained with toluidine blue. The left panel shows a dry ovary section. Vitellin granules appear light blue while the background cytoplasm of stage I, II and early stage III oocytes appears dark blue. The right panel shows the same section after adding water and a coverslip. Vitellin granules appear lavender, making them easier to distinguish from the blue cytoplasm in early stage III oocytes. Species: *Chlorurus japanensis 100x).*

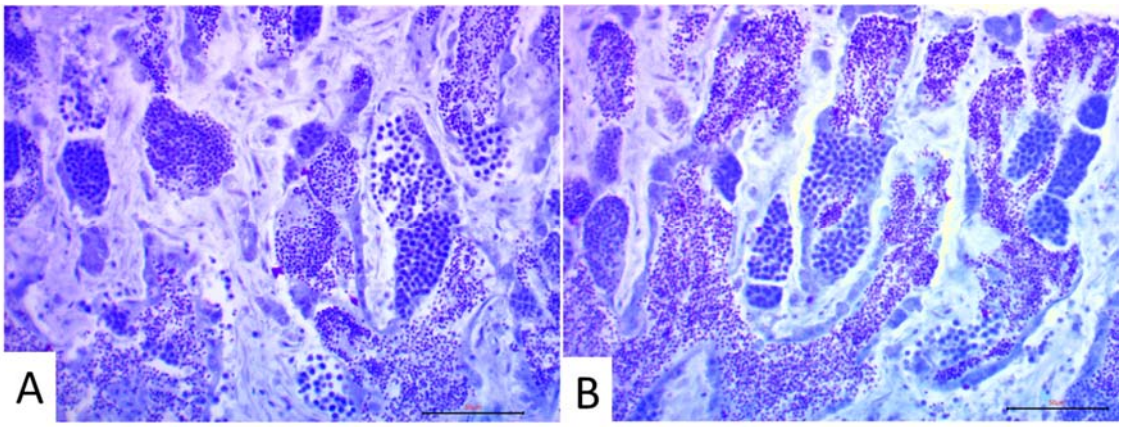


Figure 31. Effects of wet cover-slipping on a testis section stained with toluidine blue. Dry (A) and wet sections (B) are included for comparison. In wet sections, the nuclei of spermatozoa appear purple while spermatocysts remain blue. Longitudinal section. Species: *Acanthurus lineatus*, 400x.

Hematoxylin and Eosin Staining

Hematoxylin and eosin (H & E) staining uses two dyes, each with a different affinity for gonad structures. Hematoxylin has a strong affinity for nucleic acids; it stains cell nuclei light purple and nucleoli dark purple. It also stains the cytoplasm of stage I-II oocytes dark purple. Conversely, eosin has a strong affinity for proteins; it stains vitellin granules and the zona radiata a bright orange or pink and imparts a lighter pink to connective tissues. It also helps to highlight flagella so that spermatozoa can be more easily distinguished from spermatids (both are approximately the same size).

Because staining with H & E can make it easier to visualize vitellin and flagella, the technique helps to differentiate between immature and mature females (Figure 32) and males (Figure 33). However, the staining process is more complicated and costly than staining with toluidine blue. Currently, we do not teach H & E staining as a part of Jungle Histology training; however, one of many H & E protocols is detailed below. The protocol uses fewer reagents than other H & E methods, making it less cumbersome for field-based histological studies.

Stage II and III Oocytes

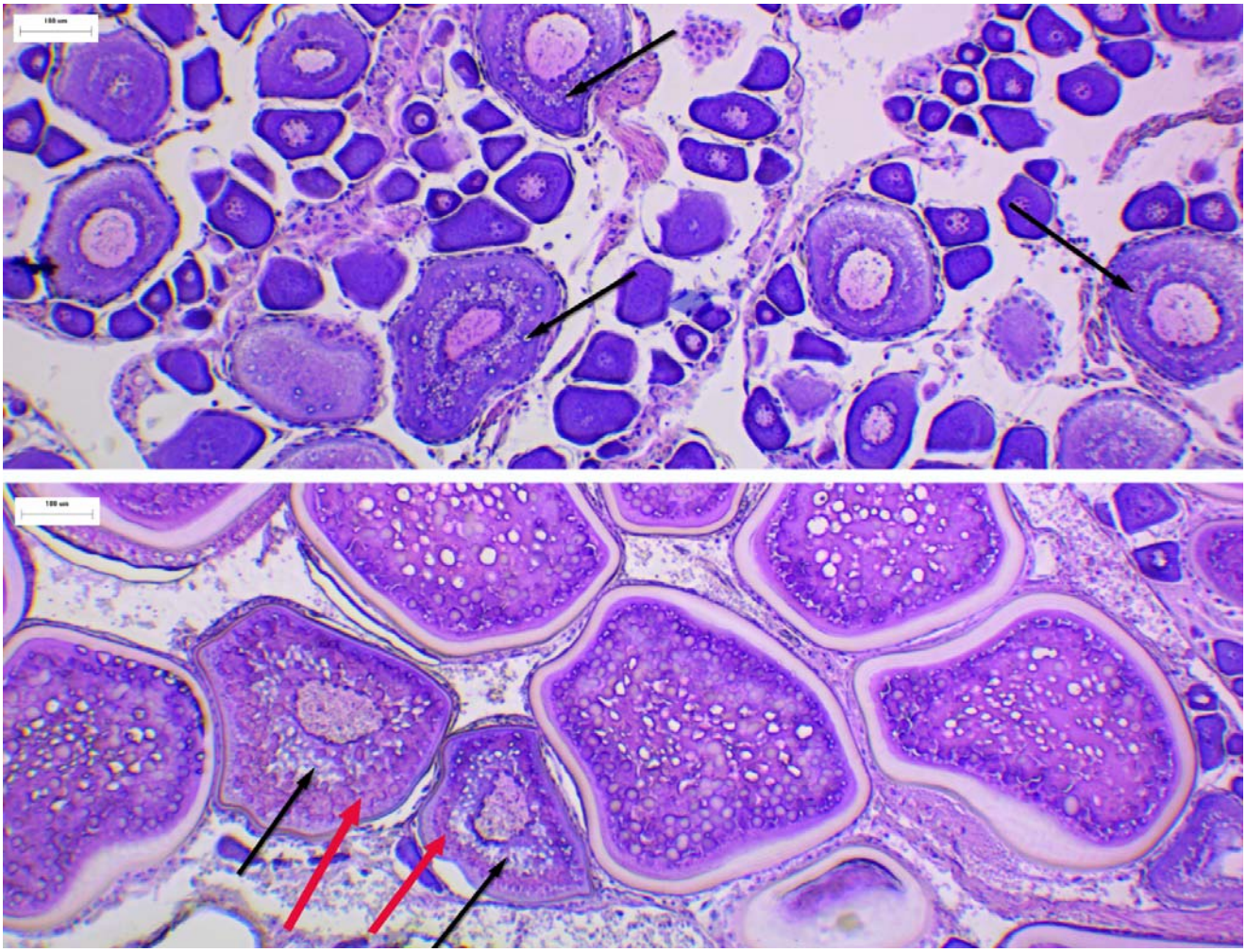


Figure 32. Hematoxylin-and-eosin-stained sections of an immature (top) and mature (bottom) ovary. Top panel shows ovary with late stage II oocytes. Bottom Panel shows ovary with early and late stage III oocytes. Black arrows indicate lipid vesicles (present in stages II and III) and red arrows indicate vitellin globules (present in stage III and IV only). Magnification; 100X. Species: *Monotaxis grandoculis* (top), *Acanthurus lineatus* (bottom).

Immature and Mature Testes

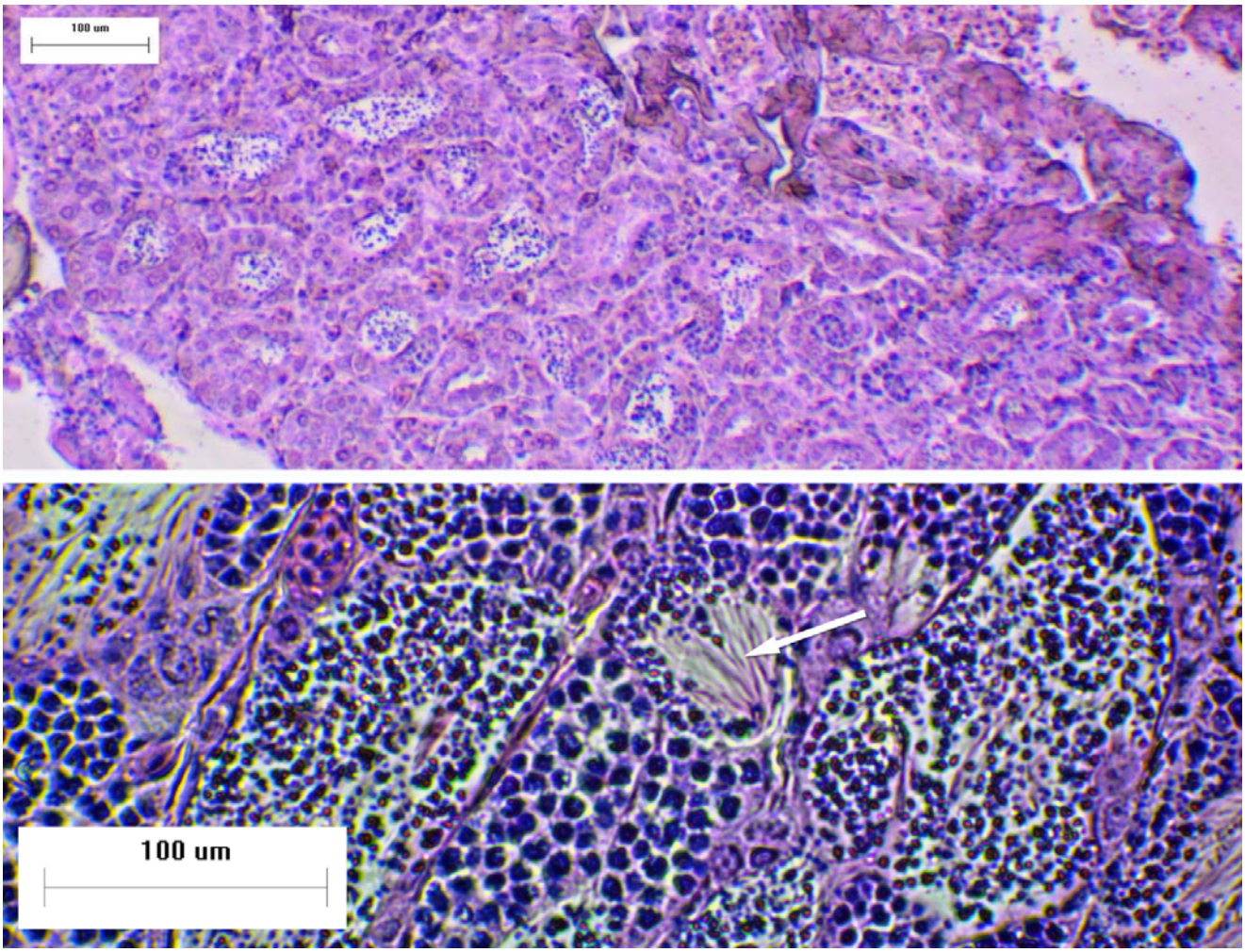


Figure 33. Hematoxylin-and-eosin-stained sections of an immature (top) and mature (bottom) testis. Nuclei stain preferentially with hematoxylin (purple) whereas cytoplasm stains mostly with eosin (pink). Eosin also helps to highlight flagella (arrow). *Acanthurus nigricauda*, 165X (top); *Lutjanus gibbus*, 400X (bottom).

The Jungle Histology Approach to H & E Staining

Commercially available, water-based hematoxylin and eosin stains have simplified H & E staining to the point that the protocol can be adapted to a Jungle Histology approach. Because of the increased number of steps and increased time required, we highly recommend processing slides using an inexpensive, plastic slide rack.

Supplies:

- 1 plastic 25-slot slide rack
- 5 plastic food-storage containers just large enough to fit the slide rack
- Shandon™ Instant Hematoxylin
- Shandon™ Instant Eosin-Y Aqueous
- 4 1-L bottles of distilled water
- ~5% solution of acetic acid (distilled vinegar)
- Sodium bicarbonate (baking soda)
- Magnesium sulfate (Epsom salt)
- A source of running fresh water

Prepare stock solutions:

1. Hematoxylin - Using a 1-L bottle of distilled water, mix instant hematoxylin according to manufacturer's instructions. Store in water bottle (labelled hematoxylin).
2. Eosin - Using a 1-L bottle of distilled water, mix instant eosin according to manufacturer's instructions. Store in water bottle (labelled eosin).
3. Acid water - Using as much distilled water as necessary (from a 1-L bottle), prepare acid water by diluting distilled vinegar to a 1 % acetic acid content (assuming your vinegar contains the standard, 5% acetic acid, mix 200 ml vinegar with 800 ml distilled water). Store in water bottle (labelled acid water).
4. Scott's tap-water substitute – add 3.5 g baking soda and 20 g Epsom salt to a 1-L bottle of distilled water. Store in water bottle (labelled Scott's tap-water substitute).

Prepare five staining dishes (add enough solution to a plastic food-storage container such that it will cover a single slide loaded into the slide rack, but not so much that the solution will overflow a container holding a fully loaded slide rack):

1. hematoxylin solution
2. eosin solution
3. acid water
4. Scott's tap-water substitute
5. Leave the 5th plastic food-storage container empty. It will be used to hold the slide rack during the running-water steps (below).

Stain slides

- Immerse slides in prepared hematoxylin solution for 8 minutes
- Rinse slides under running fresh water for 2 minutes
- De-stain slides with acid water for 20 seconds
- Rinse slides under running fresh water for 1 minute
- Immerse slides in Scott's tap-water substitute for 45 seconds
- Rinse slides under running fresh water for 3 minutes
- Immerse slides in prepared eosin solution for 1 minute
- Rinse slides under running fresh water for 5 minutes

GLOSSARY

Atresia – The degeneration of an oocyte.

Chorion – See zona radiata.

Cross-section – See transverse section.

Cytoplasm – The contents of a cell between the cell membrane and nucleus.

Egg – See ovum.

Flagellum (plural: flagella) – A whip-like structure that enables a sperm cell to swim.

Follicle – 1. A ring of tissue surrounding the oocyte. The follicle consists of an external ring of thecal cells and internal ring of follicular cells. 2. An oocyte and its surrounding cell layers (see ovarian follicle).

Follicular cells – A single layer of squamous (thin and flat) cells immediately surrounding an oocyte.

Gamete – A mature reproductive cell; sperm or ovum (egg).

Gametogenesis – The process by which a gonial cell differentiates into a gamete.

Germinal vesicle – See nucleus (for Jungle Histology purposes). The formal definition is: the nucleus of an oocyte that is arrested in prophase of meiosis I. The germinal vesicle is present in Stage I oocytes and breaks down prior to ovulation (as meiosis proceeds).

Gonial cell – Stem cells that can differentiate into gametes. Stem cells are undifferentiated cells that can divide to produce more copies of themselves; some of these copies can differentiate into specialized cells (such as gametes).

Granulosa cells – See follicular cells.

Hydration – The rapid swelling, via fluid uptake, of a maturing oocyte just prior to ovulation. This process make eggs buoyant in seawater, and is especially pronounced in marine teleosts that release their eggs into the water column.

Lamella (plural: lamellae) – A thin, plate-like extension (appearing finger-like in longitudinal section) extending from the wall toward the center of the ovary, containing oogonia and oocytes of various stages.

Leydig Cell: Cell in the male gonad responsible for producing testosterone in response to gonadotropic hormones produced by the anterior pituitary.

Lipid vesicles – Small, spherical structures located in the cytoplasm of oocytes and containing the oily constituent of yolk.

Lobule – A blind-ended tube originating near the periphery of the testis, extending toward the center of the testis, and opening into an efferent duct (which ultimately leads to the exterior of the fish). In cross-section, lobules appear as a ring of spermatocysts surrounding an internal lumen. Lobules are the functional units of teleost testes.

Longitudinal section – A section that is cut along any plane parallel to the longest axis of a structure.

Lumen (plural: lumina) – The open space inside a tubular structure.

Nucleolus (plural: nucleoli) – Dark-staining spot(s) within the nucleus of an oocyte (for Jungle Histology purposes); these are the sites of ribosomal ribonucleic acid (RNA) synthesis.

Nucleus (plural: nuclei) – Typically the largest organelle seen in a cell. It is often centrally located. It contains the majority of the cell's genetic material (in the form of chromosomes).

Oocyte – A female germ cell of any stage prior to ovulation.

Oil droplets – See lipid vesicles.

Oil globules – Large concentrations of lipids in the cytoplasm of oocytes. One or two oil globules are formed during maturation when lipid vesicles coalesce.

Oogenesis – The process by which oogonia differentiate into ova (eggs).

Oogonium (plural: oogonia) – A gonial cell capable of differentiating into an oocyte.

Organelle – Any of the structures, with a specialized function, in cytoplasm.

Ovarian follicle – A single oocyte completely surrounded by a single layer of follicle cells and a mixed layer of thecal cells.

Ovum (plural: ova) – A female gamete that is capable of being fertilized. Upon being extruded from the surrounding follicular cell layers and into the ovarian lumen, an oocyte becomes an ovum.

Polyspermy – The fertilization of an egg by more than one sperm. The result is usually an inviable zygote.

Protandry – The sexual pattern in which an individual changes sex from male to female (adjective: protandrous)

Protogyny – The sexual pattern in which an individual changes sex from female to male (adjective: protogynous).

Sequential Hermaphrodite – A species that changes sex (for Jungle Histology purposes). More formally, an individual that functions as male and female, either sequentially or simultaneously, during its lifetime (adjective: hermaphroditic).

Sertoli Cell: A somatic cell within the testis that encapsulates germ cells of equal development to form a spermatocyst. Also known as lobule boundary cells.

Spermatocyst – A generic name for an encapsulated clump of cells at the same stage of spermatogenesis. Spermatocysts consist of germ cells (spermatocytes) encapsulated by a Sertoli cell.

Spermatid – A late product of spermatogenesis. Spermatids are formed from spermatocytes, and can develop into spermatozoa without further cell division. Spermatids do not have flagella (tails).

Spermatocyte – The products of early spermatogenesis. Spermatocytes are formed from spermatogonia and differentiate into spermatids. Spermatocytes do not have flagella (tails) and are typically larger than spermatogonia.

Spermatogenesis – The process by which spermatogonia differentiate into spermatozoa.

Spermatogonium (plural: spermatogonia) – A gonial cell capable of differentiating into spermatocytes.

Spermatozoon (plural: spermatozoa) - Sperm. A mature, motile male gamete. Spermatozoa have flagella (tails).

Spermiogenesis – The maturation of spermatids into tailed spermatozoa.

Squamous – A thin and flat structure (from Latin, meaning “like the scales of a fish or serpent”).

Teleost – The largest group of fishes (96% of all species). Most fishes on coral reefs are teleosts.

Thecal cells – A layer of cells encapsulating an oocyte and its follicular cells.

Transverse section – A section that is cut along any plane perpendicular to the long-axis of a structure (a cross-section).

Urogenital pore – An opening to the outside of the body shared by the excretory and reproductive system. Ducts from the kidneys and gonads merge prior to the urogenital pore. Thus, urine and gametes are expelled through this opening.

Vesicle – A small, membrane-bound structure that functions to store and transport materials throughout a cell.

Vitellin – The main protein constituent of yolk. It is only produced in mature females.

Vitellin globules – Liquid spheres of yolk protein, or vitellin, in the cytoplasm of an oocyte. In most teleosts, vitellin accumulates in globules; however, in some teleosts, vitellin is instead found in the form of crystalline yolk granules.

Vitelline envelope – see zona radiata.

Vitellogenesis – The process by which an oocyte accumulates the nutritional reserves that will be needed by a developing embryo.

Yolk – The material in an ovum that will provide nutrition to a developing embryo. From a Jungle Histology perspective, the two most important components are lipids (in the form of lipid vesicles and oil globules) and proteins (in the form of vitellin).

Yolk globules – see vitellin globules.

Zona pellucida – see zona radiata

Zona radiata – The “eggshell” of a teleost fish oocyte. This acellular protein layer surrounding the oocyte plays a role in preventing polyspermy and protects the developing embryo from mechanical injury.

Zygote – A fertilized ovum.

ADDITIONAL RESOURCES

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