A histological atlas of female winter flounder (*Pseudopleuronectes americanus*) oogenesis

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Introduction. The patterns of winter flounder reproductive maturation and seasonality are relatively well understood. Nonetheless, detailed images of winter flounder oogenesis have not been published, which impedes comparisons between studies, labs, etc. We organize here an atlas of oogenesis, beginning with oogonial proliferation and ending with ovulation, including oocyte degradation. Two staining methods were compared; the standard Hematoxylin & Eosin (H&E) stain and the less commonly used Schiffs-Mallory Trichrome (SMT) stain.

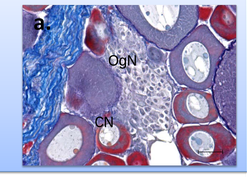
Oocyte Development and Degredation

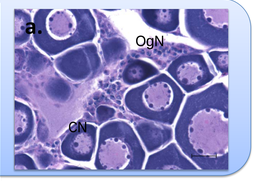
Schiffs-Mallory Trichrome (SMT)

Hematoxylin & Eosin (H&E)

Initiation: Oogonial Proliferation and Folliculogenesis

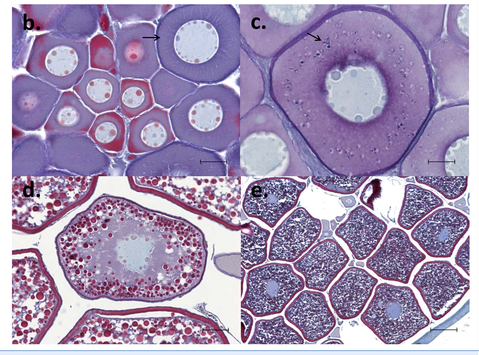
Germ cells begin as diploid (2n) oogonia followed by a reduction division, creating the oocyte (1n) which becomes enveloped in a follicle layer (folliculogenesis). We observed mitotic divisions of oogonia which produced oogonial nests (OgN) along the germinal epithelium. Chromatin nucleolar (CN) oocytes were identified as having a large nucleus.

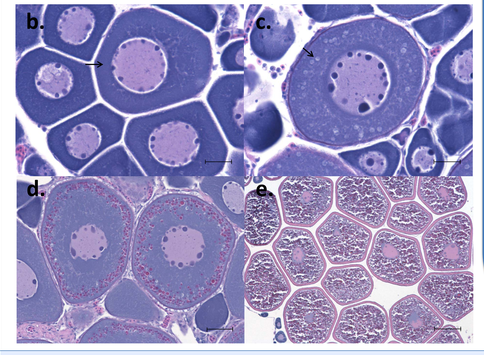




Primary and Secondary Oocyte Growth:

In these two stages germ cells grow rapidly in size and cytoplasm inclusions arise. We observed primary growth oocytes as perinucleolar (arrow, b) cells, which were present in both immature and mature fish. The key characteristic indicating the initiation of a developing fish histologically was the presence of cortical alveoli (arrow, c) in the cytoplasm, followed by vitellogenesis which marked secondary growth. Yolk inclusions formed first at the periphery of the oocyte (d) and advanced inward to the nucleus, completely filling the cytoplasm (e).





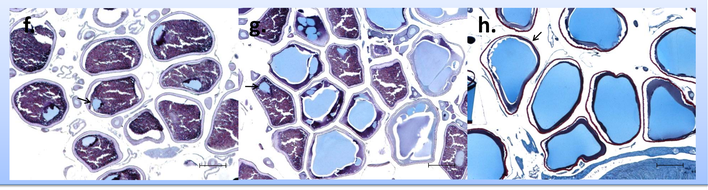
Methods

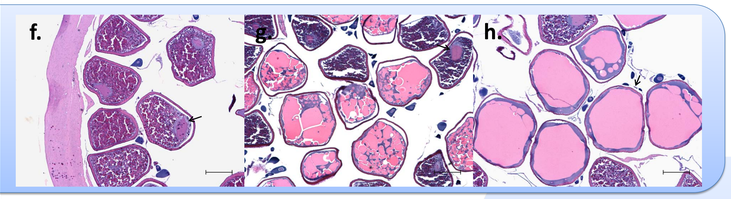
Over 500 fish were examined from four regions: Gulf of Maine, Georges Bank, southern New England, and Long Island Sound. Fresh ovary tissue was fixed in 10% buffered formalin, cut to < 1 mm thickness, and dehydrated in a series of increasing ethyl alcohol concentrations before embedding in wax. Thin sections (5 µm) of embedded tissue were stained using one of two methods: 1) Schiffs-Mallory trichrome (SMT) and 2) hematoxylin and eosin stain (H&E).

Histology slides were analyzed and images were captured using the Nikon Coolscope II. Our analysis scheme determined the most advanced oocyte stage (MAOS), the presence and stage of postovulatory follicles and atresia, presence of cysts, and tunica and stroma thickness. MAOS were defined as primary growth (all oocyte stages prior to late cortical alveolar, PG), late cortical alveolar (LC), early vitellogenic (V1), late vitellogenic (V2), germinal vesicle migration (GM), nucleus breakdown one (inside the follicle, B1), and nucleus breakdown two (outside the follicle, B2).

Oocyte Maturation:

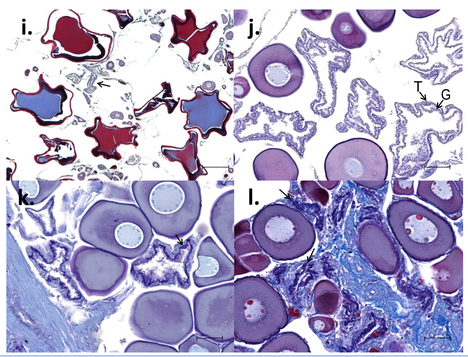
The migration of the nucleus (arrow, f, g) marks the beginning of oocyte maturation in a ripening fish. Oocytes begin to hydrate (g) as the nucleus is broken down (h), while continuing to remain in the follicle (arrow, h). We observed this entire process from the migration to the breakdown of the nucleus.

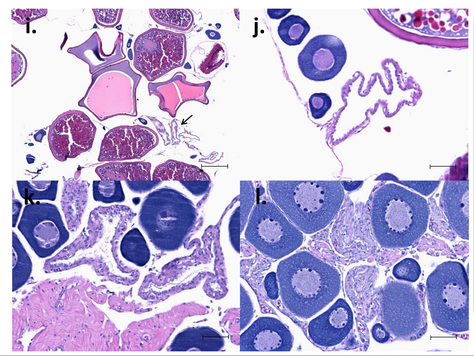




Ovulation:

Hydrated oocytes (i) outside the follicle (arrow, i) signify ovulation and a ripe & running fish. Postovulatory follicles (POFs) remain in the ovary following ovulation and egg release, and were evident in spent fish. We observed three stages of POFs: newest, older, and oldest. The newest POFs were larger than perinucleolar oocytes and the lumen was still expanded. In the SMT stain the granulose (G, j) and thecal (T, j) layers were clearly observable. Older POFs were approximately the size of a perinucleolar oocyte and the lumen was more collapsed. In the SMT stain the two layers were more distinct in color, with the granulose layer turning dark purple (arrow, k). Oldest POFs were generally smaller than perinucleolar oocytes and the lumen was very collapsed or absent. In the SMT stain the color was almost entirely dark purple (arrows, l).



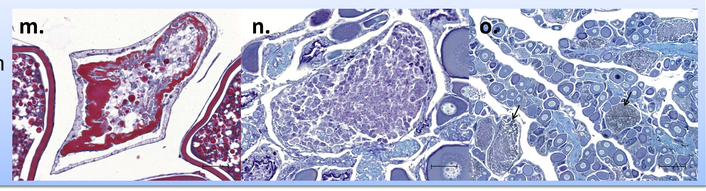


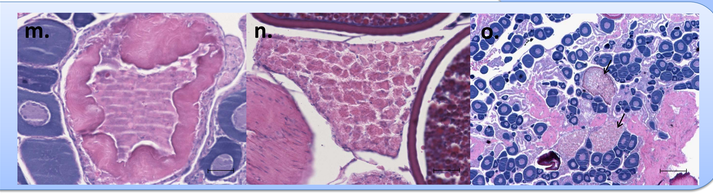
Conclusions

- Both stains illustrated the developmental progression observed in winter flounder oocytes and were consistent with previous knowledge of the species.
- The advantage of the Schiffs-Mallory Trichrome stain was most evident in post-spawning fish. The SMT stain, particularly the contrasting purple color in older postovulatory follicles (POFs), offered an advantage in identifying and staging POFs.
- This detailed characterization of developmental stages and degradation will be our standards for determination of maturity classes, verification of at-sea classifications, and estimation of maturity of ogives.
- This scheme helps identify females suitable for estimating fecundity and for identifying skip spawners (i.e., mature fish not spawning in a particular year), which are important for assessing reproductive output independently of estimates of the reproductive potential of these stocks.

Atresia:

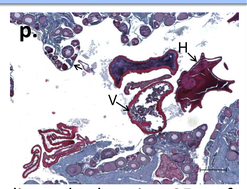
Small numbers of atretic oocytes can be observed in immature and mature fish at anytime. Alpha atresia is distinguished by the breakdown of the chorion (m), which is followed by beta atresia (n), identified by a more thorough breakdown of the whole oocyte. We occasionally observed a pronounced appearance of vitellogenic oocytes undergoing atresia, but massive atresia was rare (arrows, o).

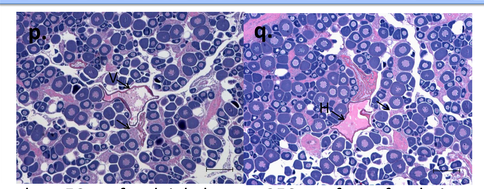




Encysted oocytes:

Oocytes that do not fully develop or ovulate can become encysted. We observed small amounts of vitellogenic (V, p) and hydrated (H, SMT p & H&E q) oocytes that were degrading in spent fish with POFs (arrow, p, q).





Scale bar applies to both stains: 25µm for a, b, c, 50µm for d, j, k, l, m, n, 250 µm for e, f, g, h, i, o, p, q.

Acknowledgements. This study was funded by NOAA's Cooperative Research Program (CRP). Most samples were provided by the CRP's Study Fleet and Industry Surveys. Supplemental samples were provided by fisheries-independent monitoring programs of Massachusetts, Rhode Island, Connecticut, University of Rhode Island, and the Northeast Fisheries Science Center. We thank in particular: K. Gottschall (CT-DEP), J. Hoey (NEFSC-CRP), J. King (MADMF), J. Moser (NEFSC-Study Fleet), R. Johnston (NEFSC-CRP), J. Collie & A. Malek (URI), E. Towle (NEFSC-PBB), J. Burnett (NEFSC-PBB), J. Dayton (NEFSC-PBB), C. LaFleur (NEFSC-PBB). and Mass Histology Service Inc.