

Seasonal patterns of oogenesis and spawning of female Yellowtail Flounder (*Limanda ferruginea*) in the Gulf of Maine: defining a period to measure potential annual fecundity.



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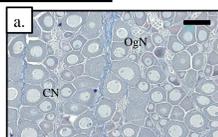
Introduction: In support of Yellowtail Flounder assessment and management, we developed a histological atlas displaying oogenesis and associated metrics of oocyte development and spawning seasonality. Yellowtail Flounder range from Newfoundland to Chesapeake Bay, with three stocks managed in the United States. This project focused on the Cape Cod/Gulf of Maine stock, where we had the most complete data set. Fish were collected primarily from commercial fishing vessels participating in cooperative research programs; additional samples acquired from fisheries-independent research surveys. As expected, oocyte development was group synchronous with respect to vitellogenesis (yolk production) and ovulation occurred in batches. These results describing the formation and ovulation of a cohort of secondary yolked oocytes indicated it is best to estimate annual fecundity from fish sampled in late winter and early spring.

Methods: A total of 731 fish were caught over a 4-year period (December 2009 – May 2012). The fish were worked up within 24 hours of being caught; body weight (0.1 g), gonad weight (0.1 g), and other biological variables were recorded. Standard paraffin histology methods were followed, staining with Schiffs-Mallory trichrome (SMT). Histology images were captured using the Nikon Coolscope II. Oocyte diameters were measured ($\pm 1 \mu\text{m}$) from digital images of histological preparations using ImageJ software. Digital images of histology were formatted to show oogenesis from oogonal proliferation to ovulation (images a-p). Similar to another study of Yellowtail Flounder gonad histology (Howell [1983] Fish. Bull. 81: 341-355), our staging criteria included: 1) most advanced oocyte stage (MAOS), 2) the presence and stage of postovulatory follicles (POF's), and 3) presence and type of atresia.

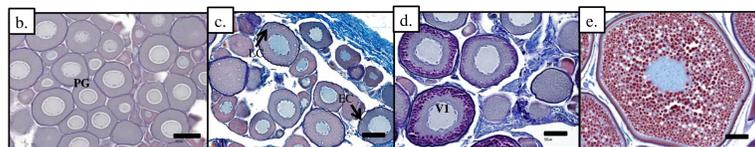
Oocyte Development and Degradation

Initiation: Oogonal Proliferation and Folliculogenesis

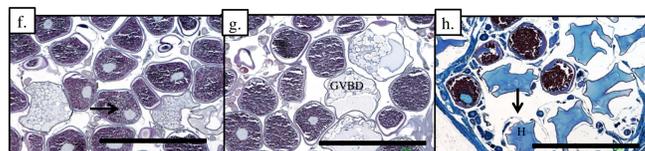
Germ cells begin as diploid (2n) oogonia followed by a reduction division, creating the oocyte (1n), which becomes enveloped in a follicle layer (folliculogenesis). We observed oogonal nests (a. OgN) and chromatin nucleolar (a. CN) oocytes which contain a large nucleus.



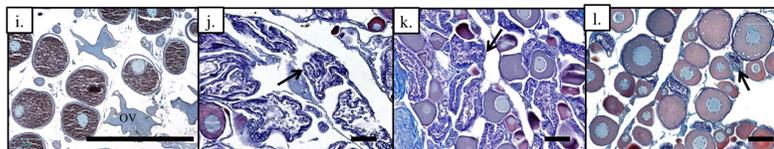
Primary and Secondary Growth Oocytes: Perinucleolar oocytes (b. primary growth, PG) are seen in immature and mature fish. Cortical alveoli which are found in the cytoplasm, indicate the start of a new annual cohort. Here it is broken into 2 categories: early cortical (c. EC, arrow indicating white inclusion in cytoplasm), followed by late cortical (c. LC, arrow indicating block dot within white inclusion). Vitellogenesis marks secondary growth. First, yolk granules form at the periphery of the oocyte (d. V1); these granules continue to advance inwards towards nucleus filling up the cytoplasm (e. V2).



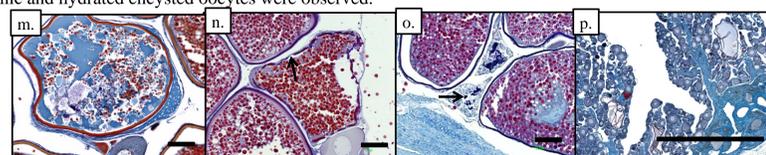
Oocyte Maturation: Germinal vesicle migration (f. GM, arrow) marks the beginning of oocyte maturation. While remaining in the follicle, the oocyte begins to hydrate as the germinal vesicle is broken down (g. GVBD). Next, the interior of the cell is completely homogeneous, no yolk globules or nucleus apparent. Oocyte is now hydrated (h. H) and still within the follicle (h. arrow). The irregular shape is due to the dehydration steps in the histology processing.



Ovulation: Once outside the follicle, ovulation (i. OV) is in process. The oocyte is officially an egg. Three stages of postovulatory follicles (POFs) are identified. POF 1 (j. arrow) are larger than perinucleolar oocytes, the lumen is very spacious, and the granulose and thecal layers are clearly visible. POF 2 (k. arrow) are similar in size to perinucleolar oocytes, the lumen is more collapsed, and the granulose layer is thickening up. POF 3 (l. arrow) are smaller than perinucleolar oocytes, the lumen is completely collapsed, and the granulose layer is visible with dark staining.



Atresia and Encysted Oocytes: Small amounts of atretic cells can be found in immature and mature fish. Alpha atresia (m, arrows and n) is identified by breaks in the zona pellucida (n. arrow) along with the collapse and breakdown of yolk globules (n). Beta atresia (o. arrow) is smaller in size than alpha and no yolk remains. Oocytes that do not fully develop or ovulate can become encysted (p. arrow); however, only a small amount of degrading vitellogenic and hydrated encysted oocytes were observed.



Scale bar for images 50 μm for a, b, c, d, e, j, k, l, m, n, o, q and 250 μm for f, g, h, i, p, r

Yellowtail Flounder have group-synchronous oocyte development.

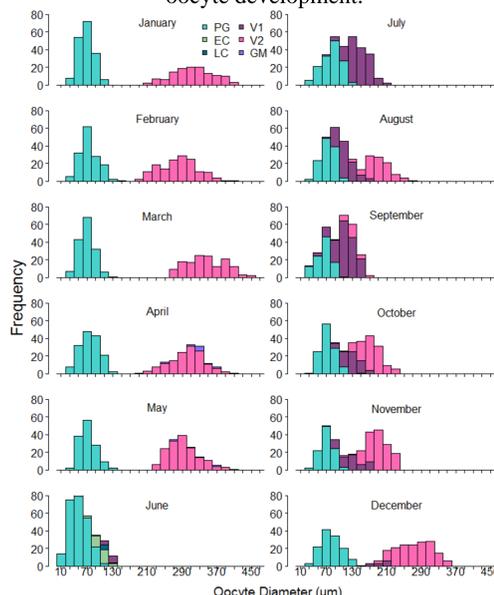


Figure 1: Diameters of ~300 oocytes sectioned through the nucleus were measured and staged: PG, primary growth oocyte; EC, early cortical alveolar oocyte; LC, late cortical alveolar oocyte; V1, primary vitellogenic oocyte; V2, secondary vitellogenic oocyte; GM, germinal vesicle migration. Each month is represented by 1 female with a GSI equal to the mean GSI for that month.

The seasonal spawning cycle was also evident from gonad weight and histology variables.

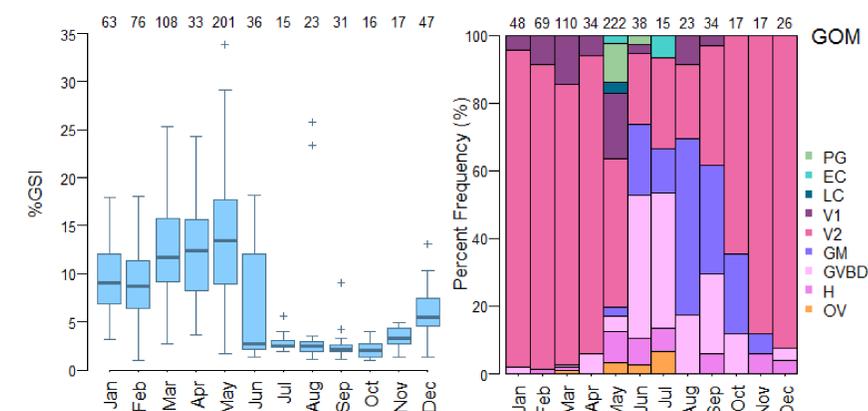
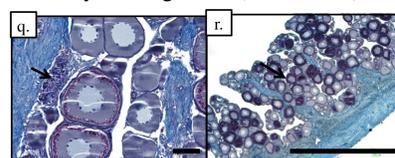


Figure 2: Box-and-whisker plot of the gonadosomatic index (GSI = gonad weight / gonad-free body weight x 100) by month for the GOM stock. The numbers above represent sample sizes.

Figure 3: Percent frequency of the most advanced oocyte stage (MAOS) determined by histology. The numbers above represent sample sizes.

Results:

- Gulf of Maine Yellowtail Flounder spawned from late spring to early summer, some spawning was evident in late summer, and generation of a new oocyte cohort occurred during the rest of the year. This seasonality was evident in all three responses: progression of oocyte diameters, gonadosomatic index (GSI), and the percent frequency of the most advanced oocyte stage (MAOS).
- Oocyte diameters (Fig. 1): A reservoir of PG oocytes existed year-round. A new cohort of secondary oocytes emerged as early as June, and a bimodal distribution was evident by late summer-early autumn. By January, a distinct hiatus for that year's clutch was visible.
- GSI (Fig. 2): Relative gonad weight increased steadily from October to May, dropped in June, and was lowest from July to October.
- MAOS (Figs. 1, 3): Active spawning is evident in late spring-early summer by the presence of mature oocytes (GM, GVBD, H, OV). Regeneration of a new oocyte cohort – to be spawned next year – occurs contemporaneously, when partially yolked stage (V1) emerges, adds yolk (V2), and grows in diameter.
- Post-spawning fish displayed evidence of atresia (r. arrow) and/or encysted oocytes (p. arrow), while at the same time 'ramping up' for the subsequent spawning season, i.e., early vitellogenesis (r. arrow, V1).



- Alpha and beta atresia of secondary growth cells were observed at low levels during vitellogenesis and the post-spawn recovery period. No evidence of mass atresia of an entire batch was seen.

Discussion:

- Spawning occurred over a broad period (May-August). Batch spawning by individuals leads to a broad and asynchronous spawning period.
- At the outset of this study, we sought to define the optimal period to estimate fecundity: once a new, distinct cohort of vitellogenic oocytes is formed (V2), but before nucleus breakdown (GVBD). Our results demonstrate it is best to estimate annual fecundity from fish sampled in late winter and early spring (March-May).
- One difficulty we encountered was the presences of small vitellogenic (V1-V2) oocytes at the end of the spawning season. Were these headed towards atresia or would they be spawned? We resolved this by examining this monthly data to demonstrate that Yellowtail Flounder "front-load" a new cohort of vitellogenic oocytes well in advance of spawning. These small vitellogenic oocytes were generating a new cohort to be spawned the following calendar year.
- Thus, during late spring and early summer, individual fish are spawning, "cleaning up" from spawning, as well as "ramping up" for the next year. Later, during the regenerating period, atresia was only observed at low levels. Large scale down-regulation of fecundity has been measured quantitatively in a companion study (McElroy *et al.*, in prep.) and is not a concern.
- The histology atlas not only defined an optimal window to measure fecundity, but it should be useful to characterize individual maturity classes and spawning frequency.

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