Beyond '*Flatland*': using gonad histology to classify female winter flounder reproductive status

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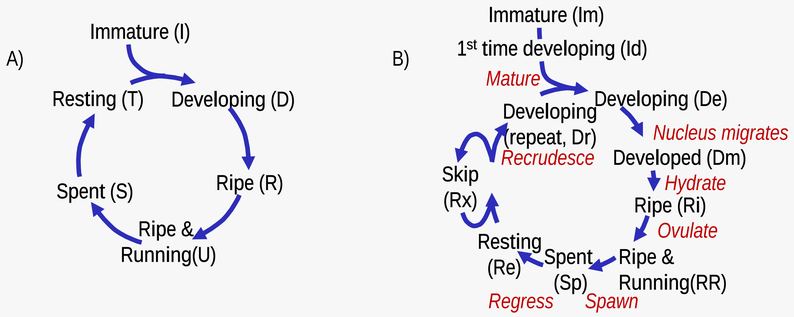
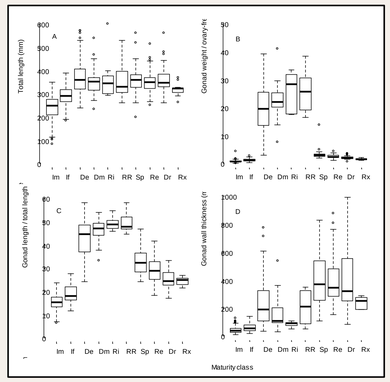
Spawning stock biomass is based on separating immature and mature components of a fishery stock. Maturity is typically identified by characters observable at sea, such as gonad size, shape, or color. How do we know these macroscopic characteristics are valid? At the NEFSC, we used gonad histology to validate these visual characters used at sea. Here, we depict a flatfish example and liken this to E. A. Abbott's book, "Flatland," where A. Square's 2-dimensional world is changed when he receives a visit from Sphere, an inhabitant of a 3-dimensional world called Spaceland. As in the book, we find that gonad histology reveals dimensions of flatfish reproduction that are unknowable from macroscopic characters. In this poster, we illustrate the dynamics of key diagnostic characters relative to each maturity class: fish size, gonad size, and gonad wall thickness (right panel) and gonad color and transitions of the germ cell itself, the oocyte (bottom panel).

Two complementary maturity schemes are introduced here: (A) A 6-class scheme used at sea when examining fresh gonads macroscopically, and (B) an expanded and nested 10-class maturity scheme with biological processes (*Italics*) inferred from gonad histology.
(A) The IDRUST scheme (http://www.nefsc.noaa.gov/nefsc/publications/tm/tmfnec76.pdf),
(B) The IDRUST+ scheme (McBride et al. 2013; doi: 10.1016/j.seares.2012.04.005).



A fish matures once but may spawn many times in its lifetime. Therefore, we expect that mature fish will be larger than immature fish, and they are (A). We also expect that gonad size will cyclically increase and decrease in size with respect to season, and it does (B, C). Finally, we expect that the gonad wall will thicken after spawning, and it does (D). These patterns verify that our histology scheme is accurate and can thereby inform the macroscopic scheme.



Each panel is a box-whisker plot, where the dark horizontal line is the median value, the outer box represents the first and third quartiles, extended bars indicate the approximate 95% confidence limits, and the individual points are outliers. All females (n = 819) were measured for total length (TL); other morphometrics were subsampled (≥ 528 females).

Summary: Macroscopic characters, seen with the unaided eye, are commonly used to assess maturity. Using these characters is inexpensive and well suited at sea. However, they are also subjective, so training is necessary to achieve satisfactory accuracy and precision. For example, this research identified confusion between Immature (I) and Resting (T) classes, and training of seagoing staff now emphasizes the differences between these classes.

Microscopic characters, using histology, are more accurate and precise. However, histology is an additional cost and laboratory processing requires 2-3 months. Although not feasible as a routine approach, gonad histology helps with QA/QC of 'at-sea' maturity classification. It also facilitates investigations of other measures of fish reproduction potential, such as skip spawning rates and annual fecundity, in relation to population size and ecosystem productivity.

Take home message: The macroscopic and microscopic characters are complementary. Together, they support the NEFSC in providing data that are both reliable and cost-effective for estimating spawning stock biomass.

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References.
McBride et al. 2013. *J Sea Res 75:41-51*. DOI: 10.1016/j.seares.2012.04.005
McElroy et al. 2013. *J Sea Res 75:52-61*. DOI: 10.1016/j.seares.2012.05.018
Press et al. (in review). *J Fish Biol*

Key to illustrations of macroscopic and histology depictions:
White grid = 1 cm^2.
TL (total length)

Class name at top.
A description of spawning activity follows.

Whole fish are dissected to expose the gonad (ovary).

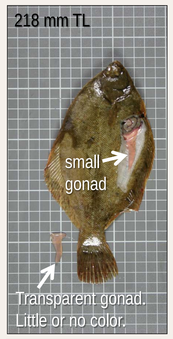
Image(s) below match gonad histology (H&E stain).

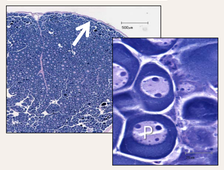
Only diagnostic traits are emphasized.

No skip spawners (Rx) are depicted. They resemble class Re but are identified near the spawning period.

Type images of immature classes:

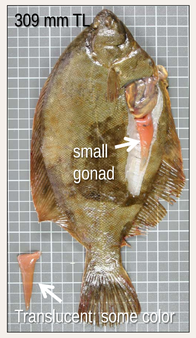
Immature (I, Im)
Never spawned.
Will not spawn > 1 yr.

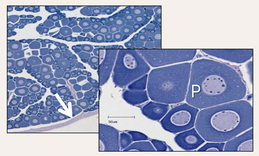




Thin gonad wall (< 100 um; arrow); pre-vitellogenic ([P]; i.e., before yolk) oocytes only. Insets: cytoplasm lightens and expands as germ cell increases in size.

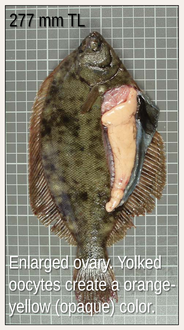
1st Time Developing (I, Id)
Never spawned.
Will spawn (1st time) ≈ 1yr.

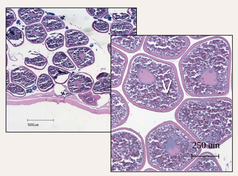




Type images of mature classes:

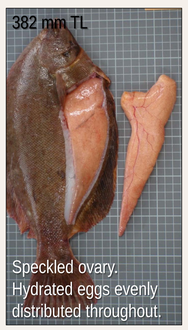
Developing (D, De, Dm)
Yolk appears.
Will spawn < 6 mo.

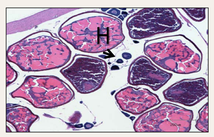




Vitellogenic oocytes (V). Inset: yolk granuales fill the cytoplasm.

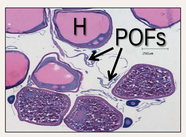
Ripe (R, Ri)
Hydration begins.
Will spawn < 1 mo.





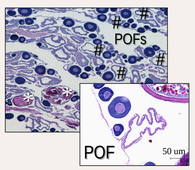
Vitellogenic (= yolked) oocytes begin to hydrate (H).

Running Ripe (U, RR)
Ovulation begins.
Actively spawning.



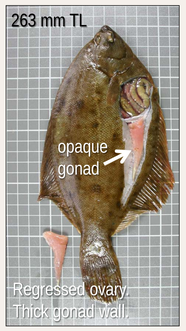
Oocytes fully hydrated. Some ovulate and collapsed postovulatory follicles (POFs) remain.

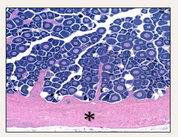
Spent (S, Sp)
Collapsed ovary.
Spawning finished.



Thick gonad wall, residual eggs (*), abundant POFs (#). Inset: detail of POF.

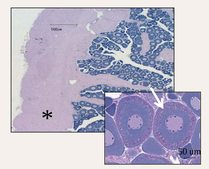
Resting (T, Re)
Has spawned, inactive now.
Will spawn ≈ 1 yr (or skip).





Thick gonad wall (0.2-1 mm; *). Only pre-vitellogenic oocytes.

Repeat Developing (T, Dr)
Has spawned; recrudescing.
Will spawn 6-12 mo.



Thick gonad wall (*). Inset: new vitellogenic oocytes (red 'yolk' inclusions, arrow).