A comprehensive description of oocyte developmental stages in Pacific halibut, *Hippoglossus stenolepis*

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**Abstract**
Accurate characterization of oocyte development is essential to understanding foundational aspects of reproductive biology and successful management of Pacific halibut (*Hippoglossus stenolepis*). Here this study provides complete histological descriptions for eight oocyte developmental stages in addition to postovulatory follicles and demonstrates the potential for oocyte size frequency distribution to act as a proxy for ovarian developmental stage and future maturity assessments. Importantly, it provides the first histological evidence that Pacific halibut have a groupsynchronous ovarian developmental pattern with determinate fecundity and support for their batch-spawning strategy.

**Keywords**
developmental stage, histology, maturity, oocyte, Pacific halibut, reproduction

Understanding the species-specific components of fish reproductive biology (e.g., age at maturity, fecundity, spawning strategy) is foundational for effective stock management. These indices vary by species (Kennedy et al., 2014; Núñez et al., 2015; TenBrink & Wilderbuer, 2015) and can dramatically alter our perception of stock status (Morgan, 2008). This is especially true for long-lived fish species, such as Pacific halibut (*Hippoglossus stenolepis*), as lifetime contributions to stock recruitment continue for many seasons after reproductive maturity is reached. For example, changes in reproductive performance, as deduced from female maturity estimates, exert a strong influence on spawning biomass estimates and, consequently, on the stock assessments of the Pacific halibut (Stewart & Hicks, 2020).

Currently, assessments of female Pacific halibut reproductive maturity involve visual macroscopic inspection of ovaries in the field (Stewart & Hicks, 2020). While convenient, this approach has yet to be corroborated by more definitive analysis methods and lacks the specificity required to provide information on many species-specific components of reproductive biology. As accurate characterizations of reproductive development are essential to fisheries management, an evaluation of the reliability of the current macroscopic staging methods using more precise assessment techniques is of utmost importance.

Histological analysis of oocyte developmental stages represents an important initial step to evaluating current maturity assessment methods (West, 1990). Moreover, when providing oocyte size-frequency distributions, histological analyses may also offer alternative methods for characterizing fish reproductive phases and offer additional information on reproductive parameters, such as fecundity type and spawning pattern. Histological examinations have successfully characterized ovarian development in many flatfishes, including California halibut *Paralichthys californicus* (Lesyna & Barnes, 2016) and Atlantic halibut *Hippoglossus hippoglossus* (Neilson et al., 1993). Furthermore, framing this characterization using universally descriptive terminology for teleost oogenesis (Grier et al., 2009) will facilitate future comparative examinations (Brown-Peterson et al., 2011).
Pacific halibut represent an important economic and cultural resource in the Gulf of Alaska and the rest of the northeastern Pacific Ocean. In this region, spawning occurs between November and March along the continental slope and in depressions on the continental shelf (St-Pierre, 1984). Spawning is generally thought to occur annually after fish reach reproductive maturity (Stewart & Hicks, 2020; St-Pierre, 1984; Thompson, 1914), but this assumption has received mixed support in the literature (Bell, 1981; Novikov, 1964; Seitz et al., 2005; Vernidub, 1936). Early investigations of Pacific halibut oocyte size (Kolloen, 1934; Thompson, 1914, 1916) documented a developing cohort of oocytes within the ovary immediately after spawning, thus supporting the premise of annual spawning. Nonetheless, a detailed histological characterization of ovarian development in female Pacific halibut to be used in histology-based reproductive phase maturity classification has not been conducted to date.

Here, this study presents comprehensive histological descriptions of oocyte developmental stages and documents postovulatory follicles (POFs) in Pacific halibut. In addition, it details the range of oocyte diameters among developmental stages and explores differences in the size-frequency distributions of oocytes in ovarian tissue at different developmental stages to investigate the relation between oocyte size and oocyte developmental stages. This work provides the most precise assessment of developmental stage for the species to date, documents the spawning strategy and offers a foundation for more specific assessments of Pacific halibut reproductive biology in the future.

### TABLE 1  Description of oocyte developmental stages of Pacific halibut, *Hippoglossus stenolepis*, associated growth phases (modified from Brown-Peterson et al., 2011 and Grier et al., 2009), and postovulatory follicles (POFs)

| Growth phase (acronym) | Developmental stage (acronym) | Description | Sample size | Mean ± S.D. | Range (min–max) |
|------------------------|-------------------------------|-------------|-------------|-------------|-----------------|
| Primary growth (PG)    | One nucleolus (PGon)          | Oocytes are small, angular and compact with a single large nucleolus. Cytoplasm granules stain dark purple. | 51          | 116 ± 89    | 36–381          |
|                        | Perinucleolar (PGpn)          | Oocytes are larger and rounder than PGon. Nuclei develop and flatten around the nucleus. Cytoplasm granules stain light purple. | 55          | 235 ± 92    | 103–479         |
|                        | Cortical alveolar (CA)        | First cortical alveoli appear as white stain in the periphery of the oocyte. | 237         | 445 ± 80    | 195–664         |
| Secondary growth (SG)  | Primary vitellogenesis (Vtg1) | Yolk globules first appear at the periphery, stain pink and fill inwards occupying up to one-third of the cytoplasm. | 663         | 544 ± 69    | 362–750         |
|                        | Secondary vitellogenesis (Vtg2)| Yolk globules transition from only the periphery of the ooplasm and fill inwards to the nucleus. | 341         | 686 ± 91    | 465–910         |
|                        | Tertiary vitellogenesis (Vtg3) | Yolk globules completely fill the ooplasm to the central nucleus and coalesce into larger yolk globules. | 500         | 1171 ± 216  | 706–1644        |
| Oocyte maturation (OM) | Germinal vesicle migration (GVM) | The nucleus begins to migrate through a cytoplasm fully filled with large yolk globules. | 302         | 1271 ± 257  | 811–1769        |
|                        | Periovulatory (PO)            | Nucleus no longer visible and the yolk globules coalesce into a central yolk mass. Oocyte is still within the follicle wall. | 54          | 2037 ± 270  | 1600–2811       |
|                        | Postovulatory follicle (POF)   | Collapsed empty follicle wall remaining after a periovulatory oocyte is expelled. |             |             |                 |
To conduct this study, the authors collected approximately 30 female Pacific halibut each month from September 2017 through August 2018 (n = 356) from the Portlock region in the Gulf of Alaska using longline fishing vessels specifically chartered for sampling. Individual sampling trips occurred over 1–4 day periods. The authors focused collection efforts on larger ($\geq$ 90 cm) individuals to increase the probability of sampling postpubescent fish (Clark et al., 2009; Loher & Seitz, 2008). Once fish were on-board the fishing vessel, et al., the probability of sampling postpubescent fish (Clark et al., 2009; Selman et al., 2010). Furthermore, the presence of POFs and atretic oocytes will be further investigated in a future study evaluating seasonal or temporal changes in maturity in female Pacific halibut.

Explorations of oocyte size distributions in fish at different female developmental stages showed that Pacific halibut follow a pattern typical of fish species with group-synchronous ovarian development with determinate fecundity (Ganias, 2013; Lubzens et al., 2010). Fish in early developmental stages displayed unimodal distributions of oocyte diameters, which became increasingly right-skewed from PGon to CA (Figure 1b). This mode – generally up to 500 $\mu$m in diameter – shares similar morphological characteristics to corresponding PG oocytes described in other fish species (Grier et al., 2009; Selman et al., 1993) and was present in all female developmental stages (Figure 1b). Fish in more advanced developmental stages displayed bimodal oocyte size distributions, with increasing separation between the two modes with progressing developmental stage (Figure 1b). This second mode developed as Vtg1 oocytes further separated in size from the previtellogenic cohort at the Vtg2 developmental stage (Figure 1b). At the Vtg3 developmental stage, a hiatus was present between the two modes, effectively separating the previtellogenic mode (oocytes <350 $\mu$m in diameter) and the larger or leading cohort of vitellogenic oocytes (> 500 $\mu$m in diameter). At the GVM and PO developmental stages, oocytes in the leading cohort continued to increase in size (> 1200 $\mu$m in diameter) up until hydration (c. 2000 $\mu$m in average). The selective recruitment of oocytes from early into later (i.e., GVM and PO) female developmental stages, as shown by the gradual transition from unimodal to bimodal size distributions leading to two different populations of oocytes in the more advanced stages of ovarian development, is evidence for a group-synchronous ovarian developmental pattern with determinate fecundity in this species (Ganias, 2013; Lubzens et al., 2010). Furthermore,
although the most advanced oocytes in the GVM developmental stage represented a unique, leading mode, the leading oocytes in the PO developmental stage showed a larger range of sizes that included GVM and PO oocytes at different steps of hydration (Figure 1b). These observations suggest that, despite the lack of information of the temporal progression of these events and the relatively small sample size in the PO developmental stage, GVM oocytes may be recruited for final maturation, hydration and subsequent ovulation in batches, supporting the notion that Pacific halibut, like its Atlantic congener (Haug & Gulliksen, 1988), is a batch spawner, as suggested by a previous report of the spawning behaviour of one tagged Pacific halibut female (Seitz et al., 2005).

The histological description of oocyte developmental stages produced by this work provides an important and necessary
framework for characterizing reproductive phase and allows for future explorations of reproductive development in Pacific halibut. The authors propose future research to evaluate seasonal changes in ovarian developmental stages, as defined here, as well as reproductive phases by investigating POFs and atresia observed in conjunction with the developmental stages. After a thorough investigation of reproductive phases in Pacific halibut, the authors further recommend future research in the area of assessing the accuracy of current macroscopic reproductive maturity staging methods, with special attention to the potential identification of skip-spawning females and their assignment to particular macroscopic maturity stages. The authors believe that their results on oocyte menstruation and size-frequency distributions presented here will be important to explore potentially time- and cost-efficient alternatives to histological oocyte evaluations for reproductive assessments in this commercially important species. Oocyte measurement methods have previously been demonstrated as effective reproductive phase indicators in Atlantic halibut (Neilson et al., 1993), and newer methods (Friedland et al., 2005; Thorsen & Kjesbu, 2001; Witthames et al., 2009) with improved detection, cost and efficiency could potentially be applied to Pacific halibut. Although fecundity at size or age of Pacific halibut is a knowledge gap, it should be noted that although mean oocyte diameter has been linked to oocyte density for use in calculating fecundity (Thorsen & Kjesbu, 2001), the analysis would require additional oocyte diameter explorations to calibrate whole mount oocytes with the histology-derived oocyte diameters presented here.

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CONFLICT OF INTEREST

All authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

T.F. contributed to the study design, collected the samples, analysed and interpreted the results, and was the primary manuscript author. N.W. assisted with analysis, interpretation and presentation of the results and assisted T.F. in writing the manuscript. B.P.H. assisted with the presentation of the results and edited the manuscript. J.V.P. conceived the study, supervised data collection, analysis and interpretation, and edited the manuscript. All authors read and approved the final manuscript.

COMPLIANCE WITH ETHICAL STANDARDS

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